

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)



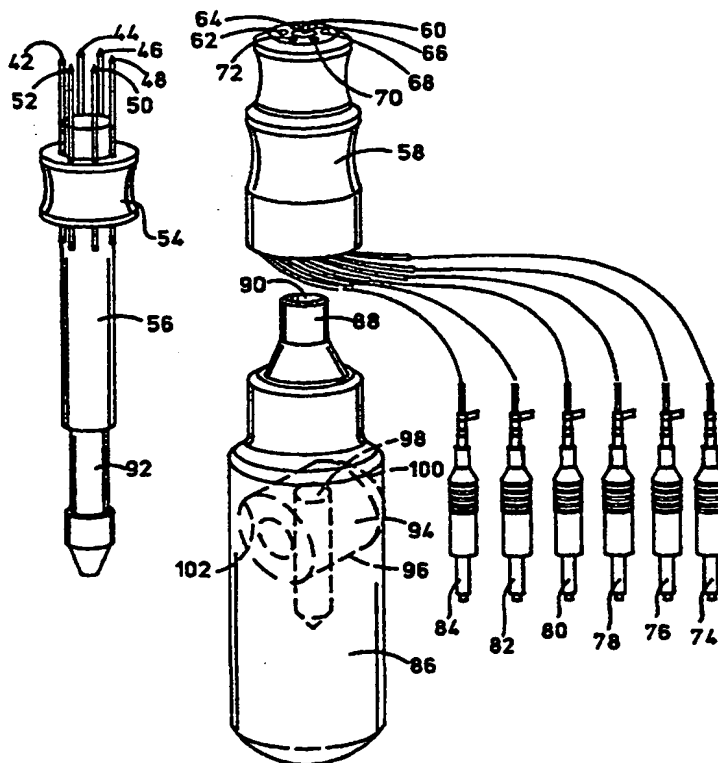
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61N 1/32		A1	(11) International Publication Number: WO 96/39226
			(43) International Publication Date: 12 December 1996 (12.12.96)
(21) International Application Number: PCT/US96/07470		(81) Designated States: AU, CA, CN, JP, KR, MX, RU, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 22 May 1996 (22.05.96)			
(30) Priority Data: 08/467,566 6 June 1995 (06.06.95) US 08/537,265 29 September 1995 (29.09.95) US		Published With international search report.	
(71) Applicant: GENETRONICS, INC. [US/US]; 11199-A Sorrento Valley Road, San Diego, CA 92121 (US).			
(72) Inventors: DEV, S., B.; 5205 Fiore Terrace #B-215, San Diego, CA 92122 (US). HOFMANN, Gunter, A.; 3750 Riviera Drive #6, San Diego, CA 92109 (US). GILBERT, Richard, A.; 10740 North 56th Street, Tampa, FL 33617 (US). HAYAKAWA, Yosuhiko; 2-2-16, Kakemama, Ichikwa City, Chiba 272-01 (JP). HELLER, Richard; 1102 Pine Ridge Circle West, Brandon, FL 33511 (US). JAROSZESKI, Mark, J.; 15501 Bruce B. Downs Boulevard #307, Tampa, FL 33647 (US).			
(74) Agent: BAKER, Freling, E.; Baker, Maxham, Jester & Meador, Suite 3100, 750 B Street, San Diego, CA 92101 (US).			

(54) Title: METHOD OF TREATMENT USING ELECTROPORATION-MEDIATED DELIVERY OF DRUGS AND GENES

(57) Abstract

A method for *in vivo* electrotherapy, or electroporation-mediated therapy, using a needle array apparatus is provided. Treatment of tumors with a combination of electroporation using the apparatus of the invention, and a chemotherapeutic agent, caused regression of tumors *in vivo*.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

METHOD OF TREATMENT USING ELECTROPORATION
MEDIATED DELIVERY OF DRUGS AND GENES

TECHNICAL FIELD

The present invention relates to the treatment of ailments in humans and other mammals, and more particularly, to an improved method and apparatus for the application of controlled electric fields for *in vivo* delivery of genes and pharmaceutical compounds into live cells of a patient by electroporation.

BACKGROUND ART

In the 1970's it was discovered that electric fields could be used to create pores in cells without causing permanent damage to them. This discovery made possible the insertion of large molecules into cell cytoplasm. It is known that genes and other molecules such as pharmacological compounds can be incorporated into live cells through a process known as electroporation. The genes or other molecules are mixed with the live cells in a buffer medium and short pulses of high electric fields are applied. The cell membranes are transiently made porous and the genes or molecules enter the cells. There they can modify the genome of the cell.

Electroporation has been recently suggested as one approach to the treatment of certain diseases such as cancer. For example, in the treatment of certain types of cancer with chemotherapy it is necessary to use a high enough dose of a drug to kill the cancer cells without killing an unacceptable high number of normal cells. If the chemotherapy drug could be inserted directly inside the cancer cells, this objective could be achieved. Some of the best anti-cancer drugs, for example, bleomycin, normally cannot penetrate the membranes of certain cancer cells. However, electroporation makes it possible to insert the bleomycin into the cells.

One therapeutic application of electroporation is for cancer treatment. Experiments on laboratory mammals have been carried out and reported as follows: Okino, M., E. Kensuke, 1990. The Effects of a Single High Voltage Electrical Stimulation with an Anticancer Drug on *in vivo* Growing Malignant Tumors. Jap.

Journal of Surgery. 20: 197-204. Mir, L.M., S. Orlowski, J. Belehradek Jr., and C. Paoletti. 1991. Electrochemotherapy Potentiation of Antitumor Effect of Bleomycin by Local Electric Pulses. Eur. J. Cancer. 27: 68-72. Clinical trials have been conducted and reported by Mir, L. M., M. Belehradek, C. Domenge, S. Orlowski, B. Poddevin, et al. 1991. Electrochemotherapy, a novel antitumor treatment: first clinical trial. C.R. Acad. Sci. Paris. 313: 613-618.

This treatment is carried out by infusing an anticancer drug directly into the tumor and applying an electric field to the tumor between a pair of electrodes. The field strength must be adjusted reasonably accurately so that electroporation of the cells of the tumor occurs without damage, or at least minimal damage, to any normal or healthy cells. This can normally be easily carried out with external tumors by applying the electrodes to opposite sides of the tumor so that the electric field is between the electrodes. The distance between the electrodes can then be measured and a suitable voltage according to the formula $E=V/d$ can then be applied to the electrodes (E =electric field strength in V/cm; V =voltage in volts; and d =distance in cm). When internal tumors are to be treated, it is not easy to properly locate electrodes and measure the distance between them. In the aforementioned parent application, there is disclosed a system of electrodes for *in vivo* electroporation wherein the electrodes may be inserted into body cavities. In a related U. S. Patent, No. 5,273,525 a syringe for injecting molecules and macromolecules for electroporation utilizes needles for injection which also function as electrodes. This construction enables the subsurface placement of electrodes. It would be desirable to have an electrode apparatus having electrodes that can be inserted into or adjacent tumors so that predetermined electric fields can be generated in the tissue for electroporation of the cells of the tumor.

Studies have also shown that large size nucleotide sequences (up to 630 kb) can be introduced into mammalian cells via electroporation (Eanault, *et al.*, *Gene (Amsterdam)*, 144(2):205, 1994; *Nucleic Acids Research*, 15(3):1311, 1987; Knutson, *et al.*, *Anal. Biochem.*, 164:44, 1987; Gibson, *et al.*, *EMBO J.*, 6(8):2457, 1987; Dower, *et al.*, *Genetic Engineering*, 12:275, 1990; Mozo, *et al.*, *Plant Molecular Biology*, 16:917, 1991), thereby affording an efficient method of gene therapy, for example.

DISCLOSURE OF INVENTION

Accordingly, it is a primary object of the present invention to provide an improved apparatus that can be conveniently and effectively positioned to generate predetermined electric fields in pre-selected tissue.

5 It is another principal object of the present invention to provide an improved apparatus that provides an effective and convenient means for positioning electrodes into tissue for the injection of therapeutic compounds into the tissue and application of electric fields to the tissue.

10 In accordance with a primary aspect of the present invention an electrode apparatus for the application of electroporation to a portion of the body of a patient, comprises a support member, a plurality of needle electrodes adjustably mounted on said support member for insertion into tissue at selected positions and distances from one another, and means including a signal generator responsive to said distance signal for
15 applying an electric signal to the electrodes proportionate to the distance between said electrodes for generating an electric field of a predetermined strength.

Another aspect of the invention includes needles that function for injection of therapeutic substances into tissue and function as electrodes for generating electric fields for portion of cells of the tissue.

20 In yet another aspect of the invention is provided a therapeutic method utilizing the needle array apparatus for the treatment of cells, particularly tumor cells.

BRIEF DESCRIPTION OF DRAWING

The objects, advantages and features of this invention will be more readily appreciated from the following detailed description, when read in conjunction with the accompanying drawing, in which:

25 Fig. 1 is a side elevation view, in section of a needle assembly in accordance with a preferred embodiment of the invention.

Fig. 2 is a bottom view of the embodiment of Fig. 1.

Fig. 3 is an assembly drawing showing a perspective view of an alternate embodiment of the invention .

Fig. 4 is a perspective view of the embodiment of Fig. 3 shown assembled.

Fig. 5 is a perspective view of a selector switch for the electrode assembly of Fig. 4.

5 Figs. 6a-6b is a diagrammatic illustration of selected contact positions of the switch of Fig. 5.

Fig. 7 is a perspective view of a further embodiment of the invention.

Fig. 8 is a perspective view of a still further embodiment of the invention.

Figs. 9a-9d is a top plan view, illustrating a preferred form of electrodes and sequence of use.

10 Figs. 10a and 10b show the tumor volume after 43 days of ECT with bleomycin in Panc-3 xenografted nude mice. (D=drug; E=electroporation)

Fig. 11 is an illustration of tumor growth of Panc-3 cells after ECT with bleomycin in nude mice.

15 Figs. 12a and 12b show the tumor volume after 20 and 34 days of ECT with bleomycin, respectively, in non-small cell lung carcinoma (NSCLC) xenografted nude mice. (D=drug; E=electroporation)

Fig. 13 shows the tumor volume after 34 days of ECT with bleomycin in non-small cell lung carcinoma (NSCLC) xenografted nude mice. The arrow indicates retreatment of one mouse at day 27. (D=drug; E=electroporation)

20 Figs. 14a and 14b show pre-pulse dosing with neocarcinostatin in Panc-3 and NSCLC, respectively, in the nude mouse model.

Figs. 14c and 14d show post-pulse dosing with neocarcinostatin in Panc-3 in the nude mouse model.

BEST MODE FOR CARRYING OUT THE INVENTION

25 As used herein the term "molecules" includes pharmacological agents, genes, antibodies or other proteins. One human therapeutic application of electroporation consists of infusion of an anticancer drug and electroporation of the drug into the tumor by applying voltage pulses between electrodes disposed on opposite sides of the tumor, called electrochemotherapy (ECT). The present invention was devised primarily for
30 enabling ECT such as that reported by Okino and Mir et al to be carried out on non-

surface tumors such as those inside the body. However, it may be utilized for other therapeutic applications.

Referring to Fig. 1 of the drawings, a needle assembly in accordance with preferred embodiment of the invention is illustrated and designated generally by the numeral 10. The needle assembly comprises an elongated tubular support body 12 which is preferably in the form of a hollow stainless steel shaft. A center needle mount 14 is mounted on the lower end of the shaft 12 and has a central bore 16 for receiving and guiding a center needle 18. The shaft 12 includes a needle exit slot 20 through which the needle electrode 18 extends from the interior thereof to the exterior where it is secured by a clamp 22 to the outside of the tube 12.

The upper end of the electrode 18 may be secured to a screw 24 for connection to an electrical circuit. The lower end of the tubular holder 12 includes threats 26 for threadably receiving a collar 28 for mounting a plurality of needles and a stop collar 30 for stopping or locking the collar 28 in position.

A plurality of needles 32 are mounted in grooves 34 equally spaced around the outer surface of the needle collar 28. This provides a circular array of equally spaced needles, eight in number in the illustrated embodiment. The needles are held in place by a band clamp 36, having the ends clamped together by a screw or nut and bolt 38 which also serves as an electrical connection for the needles. The band clamp 36 directly engages and holds the needles in place.

This electrode assembly is designed to apply electrical energy to living tissue when the needles are inserted into the tissue. The center needle 18 acts as one electrode, such as an anode or cathode, and the other or annular arrangement of needles 32 functions as the opposite electrode. All of these needles are held in fixed positions when the clamps are installed and secured. One or more of the needles may be cannular or tubular in form for injecting molecules of genes, pharmaceutical or other substances into the tissue.

In operation the center needle should be adjusted in order to achieve the desired tissue penetration. This is done by releasing the pressure of the center needle clamp 22 and sliding the center needle 18 outwardly or inwardly, as seen in Fig. 1, so that it extends from the center needle guide 14 to desired penetration distance. The needle is

then clamped in position. Thereafter the annular needles 32 are adjusted to achieve the desired penetration into the tissue. This can be accomplished by releasing the pressure of the band clamp 36 and sliding the needles 32 into the desired position. Minor adjustments can also be made by moving the needle collar 28 toward and away from the end of the shaft 12. A therapeutic substance may be injected into the tissue through one or more of these needles or by a separate means.

After all needles are adjusted to the proper penetration, the shaft 12 is grasped and the needles are inserted into the tissue to the desired depth. Thereafter, a suitable pulse generator is connected to the electrode assembly and the appropriate voltage applied to the electrodes. A suitable quantity of therapeutic substance such as genes or molecules of a suitable chemical or pharmaceutical for treatment of the tissue is injected into the tissue before the voltage is applied.

A modification to this electrode assembly could include a solid non-penetrating electrode (not shown) in place of the center needle. The non-penetrating center electrode could be any suitable shape conductor such as a button or plate attached to the end of the shaft 12 to contact the surface tissue. The annular needle arrangement would be adjusted to penetrate the tissue at the desired depth when the center electrode is resting on a tissue surface. Electrical energy would flow from the penetrating needles through the tissue and to the central electrode on the surface. These arrangements can be utilized to treat near surface tumors where the circular array of electrodes are designed to encircle the tumor. The central electrode is positioned such that the electrical energy flows through the tumor to the central electrode.

Other advantages of this electrode assembly are that all needles 18 and 32 can be independently adjusted to achieve the desired penetration. The needle 28 collar can also be adjusted to position it from the end of the shaft 12 so that insertion of the center and annular needles can be directly observed. In addition, the needle collar 28 can have any size or configuration to encircle the tissue area to be treated.

Referring to Figs. 3 and 4 an alternate embodiment of a circular array needle electrode assembly is illustrated and designated generally by the numeral 40. This needle assembly comprises a circular array of needles 42 through 52, which are mounted in equally spaced relation in a hub 54 mounted on an elongated cylindrical shaft 56.

The hub 54 is preferably of a suitably selected diameter to provide the desired diameter of the arrays to position around a tumor or other tissue to be treated. One or more of the needles may be hollow to enable the injection of molecules of a therapeutic substance, as will be more fully described hereinafter.

5 An electrical connector socket assembly comprises a body member 58 having a central opening or bore 60 for receipt of shaft 56 and an annular array of a plurality of sockets 62 through 72 for receipt of the ends of needles 42 through 52. The sockets 62 through 72 electrically connect the needles to leads 74 through 84 which connect to a distributing switch, as will be subsequently described.

10 The electrical connector socket 58 fits onto shaft 56 with the end of the needles extending into the electrical sockets 62 through 72 for connecting to the leads 74 through 84. The shaft 56 which mounts the needle array hub 54 and the socket assembly 58 mounts onto a holder 86 adapted to be held in the hand. The holder 86 has an elongated cylindrical configuration adapted to be held in the hand for manipulation.

15 The holder 86 has a forward socket and including a forwardly extending tubular shaft 88 having a bore 90 into which shaft 56 extends while the shaft 88 extends into a bore (not shown) within the connector member 58. The shaft 56 extends into bore 90 and has an annular groove or recess 92 which is engaged by a retainer latch which comprises a transverse plug 94 in a bore 96 biased to one side and including a bore 98 in which

20 the annular slot 92 extends and is retained in the holder. A spring 102 mounted in bore 96 biases plug 94 to the latched position. The shaft 56 may be released for removal by pressing on end 100 of plug 94.

 The holder when assembled as shown in Fig. 4 may be grasped in the hand and the needles inserted into a selected tissue area. The needles 42-52 are preferably spaced

25 and positioned to surround the selected tissue of treatment. One or more of the needles 42-52, as previously explained, may be hollow to enable the injection of the desired therapeutic substance. The electrode leads 74-84 are then connected in a preferred arrangement to a rotatable switch assembly, as shown in Fig. 5, which enables the selection of opposed pairs of the needles for activation or the application of the electrical

30 potential.

The switch assembly designated generally by the numeral 104 comprises a stationary housing 106 which, in the illustrated embodiment, is generally cylindrical in configuration and in which is mounted a rotor 108 with spaced contacts 110 and 112 connected by a pair of conductors 114 and 116 to a pulse power generator 115. The rotor contacts 110 and 112 are positioned within housing 106 to engage annular contacts 118, 120, 122, 124, 126 and 128 to which leads 74-84 are connected.

Referring to Figs. 6a, b and c, the rotor 108 has an internal portion having contacts 110 and 112 each of which bridge between two contacts 118-128 to which the leads 74 through 84 are connected to connect the source of power. The internal contacts 110 and 112 rotate with the rotor 108 and can be selectively positioned in conductive relation with pairs of the internal contacts 118-128 to thereby activate opposed pairs of the needle electrodes. This enables the operator to selectively position the electrodes surrounding a selected tissue and to selectively apply the direction of the electrical field as desired for optimum treatment. The rotor 108 enables the field to be selectively generated around or across the tissue from all directions.

Referring to Fig. 7 an alternate embodiment of an electric field generating array of parallel adjustably positionable electrodes, as disclosed in the parent application, is illustrated. The electrode assembly designated generally by the numeral 130 includes a pair of spaced apart arrays 132 and 134 of conductive needle electrodes 136 and 138 mounted on a dielectric carrier or support member 140. The needle array 132 is held in a fixed clamp 142 which allows the needles 136 to be adjusted in depth relative to the support 140.

The needles 138 are mounted in a moveable clamp 146 which is adjustably mounted on support member 140 by a clamp screw 148. The needles 136 and 138 are each provided with a penetration stop 144. The gap spacing clamp screw 148 secures the clamp 146 in selected positions on the support 140. A gap spacing sensor 150 senses the distance between the needle arrays 132 and 134 and generates a signal that is sent to the pulse generator via conductor cable 152. A pulse generator is connected to the needle electrodes by means of cables 154 and 156.

Referring to Fig. 8, details of a needle holder or template for various arrangements for establishing a spaced pair or parallel arrays of needles is illustrated.

This embodiment comprises a base holder member 158 having a plurality of adjacently positioned parallel slots 160 into which selected needles 162 and 164 may be positioned in selected spaced relation. This holder may serve to mount a pair of oppositely polarized needle electrodes 162 and 164, as illustrated. These can be selectively positioned in selected space relationship to be disposed on opposite sides of a selected tissue. The needles are clamped into the slots by a clamp or plate 159. In addition, the holder may be used in combination with an additional holder for provision of multiple arrays on opposite sides of a selected tissue. The illustrated needles may be connected by conductors 166 and 168 to a suitable pulse generator.

Referring to Figs. 9a through 9d, an additional aspect of the invention is illustrated. As more clearly illustrated, the combination electrodes may take the form of separate needles 170 and 172 which may be first inserted into or beside a selected tissue area such as on opposite sides of a tumor 194 as illustrated. Thereafter the needles may be connected to a syringe or other source of molecules and used to inject a selected molecular solution into the tissue area. The needles may be non-conductive and a pair of electrodes 176 and 178, as illustrated in Fig. 9b, are selectively fed through the bore or lumen of the respective needles into the tissue, as illustrated, and thereafter the needle is removed, as shown in Fig. 9c. The electrodes 176 and 178 are each provided with an elongated insulated conductor 180 and 182 with conductive tips 184 and 186.

A pair of conductors 188 and 190 from a suitable power generator may then be connected to the ends of the conductors of the electrodes by micro clamps 192 and 194, as shown in 9d, and an electric potential applied across the electrodes. This generates a field in the tissue and electroporates the cells of the selected tissue, such as a tumor or the like. This electroporation enables the selected molecules to enter the cells of the tissue and more efficiently kill or alter the cells as desired. This form of needle and electrode may be used with any or all the above described assemblies.

These needle electrode assemblies, as above described, enable the *in vivo* positioning of electrodes in or adjacent to subsurface tumors or other tissue. While the focus of the present application has been on electrochemotherapy, the embodiment of

the subject invention may be applied to other treatments, such as gene therapy of certain organs of the body.

The nature of the electric field to be generated is determined by the nature of the tissue, the size of the selected tissue and its location. It is desirable that the field be as homogenous as possible and of the correct amplitude. Excessive field strength results in lysing of cells, whereas a low field strength results in reduced efficacy. The electrodes may be mounted and manipulated in many ways including but not limited to those in the parent application. The electrodes may be conveniently manipulated on and by forceps to internal position.

The waveform of the electrical signal provided by the pulse generator can be an exponentially decaying pulse, a square pulse, a unipolar oscillating pulse train or a bipolar oscillating pulse train. The electric field strength can be 0.2kV/cm to 20kV/cm. The pulse length can be ten μ s to 100 ms. There can be one to one hundred pulses. Of course, the waveform, electric field strength and pulse duration are also dependent upon the type of cells and the type of molecules that are to enter the cells via electroporation.

The various parameters including electric field strengths required for the electroporation of any known cell is generally available from the many research papers reporting on the subject, as well as from a database maintained by Genetronics, Inc., San Diego, California, assignee of the subject application. The electric fields needed for *in vivo* cell electroporation, such as ECT, are similar in amplitude to the fields required for cells *in vitro*. These are in the range of from 100 V/cm to several kV/cm. This has been verified by the inventors own experiments and those of others reported in scientific publications. The first *in vivo* application of pulsed electric fields in the chemotherapy field to treat tumors was reported in 1987 by Okino in Japan.

Pulse generators for carrying out the procedures described herein are and have been available on the market for a number of years. One suitable signal generator is the ELECTRO CELL MANIPULATOR Model ECM 600 commercially available from GENETRONICS, INC. of San Diego, California, U.S.A. The ECM 600 signal generator generates a pulse from the complete discharge of a capacitor which results in an exponentially decaying waveform. The electric signal generated by this signal generator is characterized by a fast rise time and an exponential tail. In the signal generator, the

electroporation pulse length is set by selecting one of ten timing resistors marked R1 through R10. They are active in both High Voltage Mode (HVM) (capacitance fixed at fifty microfarads) and Low Voltage Mode (LVM) (with a capacitance range from 25 to 3,175 microfarads).

5 The ECM 600 signal generator has a control knob that permits the adjustment of the amplitude of the set charging voltage applied to the internal capacitors from 50 to 500 volts in LVM and from 0.05 to 2.5kV in the HVM. The amplitude of the electrical signal is shown on a display incorporated into the ECM 600 signal generator. This device further includes a plurality of push button switches for controlling pulse
10 length, in the Low VM mode, by a simultaneous combination of resistors parallel to the output and a bank of seven selectable additive capacitors.

 The ECM 600 signal generator also includes a single automatic charge and pulse push button. This button may be depressed to initiate both charging of the internal capacitors to the set voltage and to deliver a pulse to the outside electrodes in an
15 automatic cycle that takes less than five seconds. The manual button may be sequentially pressed to repeatedly apply the predetermined electric field.

 Preferably, the therapeutic method of the invention utilizes a square wave pulse electroporation system. For example, the ElectroSquarePorator (T820), also available from GENETRONICS, INC., can be used.

20 Square wave electroporation systems deliver controlled electric pulses that rise quickly to a set voltage, stay at that level for a set length of time (pulse length), and then quickly drop to zero. This type of system yields better transformation efficiency for the electroporation of plant protoplast and mammalian cell lines than an exponential decay system.

25 The ElectroSquarePorator (T820) is the first commercially available square wave electroporation system capable of generating up to 3000 volts. The pulse length can be adjusted from 5 μ sec to 99 msec. The square wave electroporation pulses have a gentler effect on the cells which results in higher cell viability.

 The T820 ElectroSquarePorator is active in both the High Voltage Mode (HVM)
30 (100-3000 volts) and the Low Voltage Mode (LVM)(50-500 volts). The pulse length for

LVM is about 0.3 to 99 msec and for HVM, 5 to 99 μ sec. The T820 has multiple pulsing capability from about 1 to 99 pulses.

Mir and others have used square wave pulses for electrochemotherapy, which allows the insertion of chemotherapeutic agents into cancerous tumors. Mice were
5 injected with a low dose of bleomycin. The cancerous tumors were then electroporated resulting in the reduction or complete remission of the tumors (Mir, L.M., *Eur. J. Cancer*, 27(1):68, 1991) .

Saunders has compared the square wave with exponential decay pulses in the electroporation of plant protoplast. Square wave electroporation produced higher
10 transformation efficiency than the exponential decay pulses. He also reported that the optimization of electroporation parameters is much easier with square wave pulses since sufficient transformation efficiency can be produced over a larger range of voltages (Saunders, *Guide to Electroporation and Electrofusion*, pp.227-247, 1991).

The therapeutic method of the invention includes electrotherapy, also referred to
15 herein as electroporation-mediated therapy, using the apparatus of the invention for the delivery of macromolecules to a cell or tissue. As described earlier, the term "macromolecule" or "molecule" as used herein refers to drugs (*e.g.*, chemotherapeutic agents), nucleic acids (*e.g.*, polynucleotides), peptides and polypeptides, including antibodies. The term polynucleotides include DNA, cDNA and RNA sequences.

20 Drugs contemplated for use in the method of the invention are typically chemotherapeutic agents having an antitumor or cytotoxic effect. Such drugs or agents include bleomycin, neocarzinostatin, suramin, and cisplatin. Other chemotherapeutic agents will be known to those of skill in the art (see for example The Merck Index). The chemical composition of the agent will dictate the most appropriate time to administer
25 the agent in relation to the administration of the electric pulse. For example, while not wanting to be bound by a particular theory, it is believed that a drug having a low isoelectric point (*e.g.*, neocarzinostatin, IEP=3.78), would likely be more effective if administered post-electroporation in order to avoid electrostatic interaction of the highly charged drug within the field. Further, such drugs as bleomycin, which have a very
30 negative log P, (P being the partition coefficient between octanol and water), are very large in size (MW=1400), and are hydrophilic, thereby associating closely with the lipid

membrane, diffuse very slowly into a tumor cell and are typically administered prior to or substantially simultaneous with the electric pulse. Electroporation facilitates entry of bleomycin or other similar drugs into the tumor cell by creating pores in the cell membrane.

5 It may be desirable to modulate the expression of a gene in a cell by the introduction of a molecule by the method of the invention. The term "modulate" envisions the suppression of expression of a gene when it is over-expressed, or augmentation of expression when it is under-expressed. Where a cell proliferative disorder is associated with the expression of a gene, nucleic acid sequences that interfere
10 with the gene's expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid, ribozymes, or triplex agents to block transcription or translation of a specific mRNA, either by masking that mRNA with an antisense nucleic acid or triplex agent, or by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to
15 at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are
20 easily synthesized and are less likely to cause problems than larger molecules when introduced into the target cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289, 1988).

Use of an oligonucleotide to stall transcription is known as the triplex strategy
25 since the oligomer winds around double-helical DNA, forming a three-strand helix. Therefore, these triplex compounds can be designed to recognize a unique site on a chosen gene (Maher, *et al.*, *Antisense Res. and Dev.*, 1(3):227, 1991; Helene, C., *Anticancer Drug Design*, 6(6):569, 1991).

Ribozymes are RNA molecules possessing the ability to specifically cleave other
30 single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to

engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

5 There are two basic types of ribozymes namely. *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species.
10 Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

 The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders mediated by a particular gene or absence thereof.
15 Such therapy would achieve its therapeutic effect by introduction of a specific sense or antisense polynucleotide into cells having the disorder. Delivery of polynucleotides can be achieved using a recombinant expression vector such as a chimeric virus, or the polynucleotide can be delivered as "naked" DNA for example.

 Various viral vectors which can be utilized for gene therapy as taught herein
20 include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous
25 Sarcoma Virus (RSV). When the subject is a human, a vector such as the gibbon ape leukemia virus (GaLV) can be utilized. A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated.

 Therapeutic peptides or polypeptides may also be included in the therapeutic
30 method of the invention. For example, immunomodulatory agents and other biological response modifiers can be administered for incorporation by a cell. The term

"biological response modifiers" is meant to encompass substances which are involved in modifying the immune response. Examples of immune response modifiers include such compounds as lymphokines. Lymphokines include tumor necrosis factor, interleukins 1, 2, and 3, lymphotoxin, macrophage activating factor, migration inhibition factor, colony stimulating factor, and alpha-interferon, beta-interferon, and gamma-interferon and their subtypes.

Also included are polynucleotides which encode metabolic enzymes and proteins, including antiangiogenesis compounds, *e.g.*, Factor VIII or Factor IX.

The macromolecule of the invention also includes antibody molecules. The term "antibody" as used herein is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')₂.

Administration of a drug, polynucleotide or polypeptide, in the method of the invention can be, for example, parenterally by injection, rapid infusion, nasopharyngeal absorption, dermal absorption, and orally. In the case of a tumor, for example, a chemotherapeutic or other agent can be administered locally, systemically or directly injected into the tumor. When a drug, for example, is administered directly into the tumor, it is advantageous to inject the drug in a "fanning" manner. The term "fanning" refers to administering the drug by changing the direction of the needle as the drug is being injected or by multiple injections in multiple directions like opening up of a hand fan, rather than as a bolus, in order to provide a greater distribution of drug throughout the tumor. As compared with a volume that is typically used in the art, it is desirable to increase the volume of the drug-containing solution, when the drug is administered (*e.g.*, injected) intratumorally, in order to insure adequate distribution of the drug throughout the tumor. For example, in the EXAMPLES herein, one of skill in the art typically injects 50 μ l of drug-containing solution, however, the results are greatly improved by increasing the volume to 150 μ l. Preferably, the injection should be done very slowly and at the periphery rather than at the center of the tumor where the intertidal pressure is very high.

Preferably, the molecule is administered substantially contemporaneously with the electroporation treatment. The term "substantially contemporaneously" means that the molecule and the electroporation treatment are administered reasonably close together.

with respect to time. The administration of the molecule or therapeutic agent can at any interval, depending upon such factors, for example, as the nature of the tumor, the condition of the patient, the size and chemical characteristics of the molecule and half-life of the molecule.

5 Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers for occlusive dressings can be used to increase skin permeability and enhance antigen absorption. Liquid dosage forms for oral
10 administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending the liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents. Further, vasoconstrictor
15 agents can be used to keep the therapeutic agent localized prior to pulsing.

Any cell can be treated by the method of the invention. The illustrative examples provided herein demonstrate the use of the method of the invention for the treatment of tumor cells, *e.g.*, pancreas and lung. Other cell proliferative disorders are amenable to treatment by the electroporation method of the invention. The term "cell proliferative
20 disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. Malignant cells (*i.e.*, tumors or cancer) develop as a result of a multi-step process. The method of the invention is useful in treating malignancies or other disorders of the various organ systems, particularly, for example, cells in the pancreas and lung, and also
25 including cells of heart, kidney, muscle, breast, colon, prostate, thymus, testis, and ovary. Preferably the subject is human.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLES

The following examples illustrate the use of electrochemotherapy (ECT) of a poorly differentiated human pancreatic tumor (Panc-3) xenografted subcutaneously on the left flank of nude mice. The single treatment procedure involved injection of bleomycin (0.5 units in 0.15 ml saline) intratumorally, using fanning, as described herein followed by application of six square wave electrical pulses, ten minutes later, using proprietary needle array electrodes arranged along the circumference of a circle 1 cm in diameter. Needle array of variable diameters (*e.g.*, 0.5 cm, 0.75 cm and 1.5 cm can also be used to accommodate tumors of various sizes. Stoppers of various heights can be inserted at the center of the array to make the penetration depth of the needles into the tumor variable. A built-in mechanism allowed switching of electrodes for maximum coverage of the tumor by the pulsed field. The electrical parameters were: 1300 V/cm and 6 x 99 μ s pulses spaced at 1 sec interval.

Results showed severe necrosis and edema in nearly all the mice at the treatment site. While there was a substantial reduction in the tumor volume (after a slight initial increase due to edema) of the mice in the treated group (D+E+; D=Drug, E=Electrical field), those in the control group (D+E-) increased dramatically. Nearly complete tumor regression was observed in 90% of the mice treated by ECT after 28 days. No response was seen in 10% of the mice. A complete regression with no palpable tumor has been observed in 60% of the cases 77 days after the initial treatment. However, there was tumor regrowth in 20% of the mice 35 days after treatment but at a much slower growth rate compared to the control. This observation has been linked to incomplete treatment of large primary tumors where the needle depth was lower than the Z dimension of the tumor. Histological analysis of tumor samples showed necrotic tumor cell ghosts in D+E+ group compared to a mixture of viable and necrotic cells in D+E- group. Preliminary studies with human non-small cell lung cancer (NSCLC) tumors xenografted onto nude mice have also shown very encouraging results with ECT treatment with bleomycin.

Example 1:

The tumor cell line Panc-3, a poorly differentiated adenocarcinoma cell line of the pancreas, was supplied by AntiCancer, Inc., San Diego. For ECT experiments, tissue taken from the stock mice, where the tumor line was maintained, was thawed and cut into very small pieces about 1 mm each, and 8-10 pieces were surgically xenografted in a subcutaneous sac made in left flank of nude mice, and then closed with 6.0 surgical suture. After the average tumor size reached about 5 mm, mice with palpable tumors were divided randomly, 10 mice for control group (D+E-; D=Drug, E=Electric field) and 10 mice for ECT treatment, namely bleomycin injection followed by pulsing (D+E+) from a BTX Square Wave T820 Generator. The tumor dimensions were measured and the tumor volume calculated using the formula:

$$(4\pi/3) \times a \times b \times c$$

where a, b, and c are, respectively, the length, width and thickness of the tumor. 0.5 units Bleomycin (Sigma Chemicals) was dissolved in 0.15 ml of 0.9% NaCl and was injected in each mice intratumorally by fanning for both the control (D+E-) and the treated (D+E+) groups. Ten minutes after the injection, each mouse in the D+E+ group was pulsed from a BTX T820 square wave electroporator with a set of needle array electrodes as described in the present invention. Electrical parameters used were as follows: field strength 1300 V/cm, 6 pulses of 99 μ s each, at 1 sec interval.

The mice were monitored every day for mortality and any signs of a diseased state were noted. The tumor dimensions were measured at regular intervals and tumor growth regression/progression monitored. Another set of nude mice with xenografts of non-small cell lung cancer line was also treated by the same procedure as for the Panc-3 tumors.

Figures 10a and 10b show the analysis of the tumor volume determined over a 43 day period after ECT using bleomycin for the Panc-3 tumors. There was a dramatic difference between the untreated and treated mice in terms of tumor volume. There was essentially no detectable tumor after approximately 24 days of treatment. The results of Figure 10 are also summarized in Table 1 below. An illustration of the actual regression of the tumor is shown in Figure 11.

TABLE 1
ELECTROCHEMOTHERAPY OF PANC-3 TUMORS IN NUDE MICE

Days after treatment	Tumor volume (mm ³) C1	Tumor volume (mm ³) C2	Tumor volume (mm ³) T1	Tumor volume (mm ³) T2
0	138.746	148.94	123.11	178.37
1	206.979	179.82	210.95	252.72
8	394.786	451.787	104.55	211.11
15	557.349	798.919	113.21	226.966
18	939.582	881.752	161.73	246.91
24	1391.057	1406.98	41.56	47.2228
28	1628.631	1474.21	0	0
35	2619.765	2330.31	0	0
38	2908.912	2333.967	0	0
43	3708.571	5381.759	0	0

Cell Line: poorly differentiated human pancreatic tumor (panc3)
 Mouse model: nude mouse
 Transplant: subcutaneous xenograft

Control mice: C1 and C2
 Treated mice: T1 and T2

The Panc-3 experiment was repeated using a non-small cell lung cancer cell line (NSCLC), 177 (AntiCancer, San Diego, CA). The results were similar to that found with bleomycin and Panc-3 as shown in Figures 12a and 12b. In one experiment, a tumor that had recurred was retreated at day 27 (Figure 13) and after 7 days, there was no evidence of tumor.

The Panc-3 and NSCLC models were utilized with the drug neocarcinostatin (NCS) following the same procedures as outlined above. As shown in Figure 14a and 14b, pre-pulse dosing with NCS in a manner similar to that used for the bleomycin studies, was not effective in reducing tumor size at all. It was believed that due to the low isoelectric point of NCS, electrostatic interaction prevented the drug from entering

the tumor cell. Therefore, the experiment was repeated by pulsing first and injecting NCS post-pulse (pp).

Figure 14c shows the initial tumor volume (I) as compared to the final tumor volume (F) at day 13 for 7 mice treated (Mouse ID 1-7). In several of the mice (ID 1, 2, 4, and 7), an increase in tumor volume was observed, but appeared to be due to edema. However, as shown in Figure 14d, when a separate group of 5 mice were examined at day 23, all mice showed a marked reduction in tumor volume.

A comparison of Figures 14 a and b with 14 c and d indicated that post-pulse with NCS was more effective than pre-pulse administration for NCS.

Summary

The present Examples illustrate that a poorly differentiated Pancreatic cancer (Panc-3) and Non-small cell lung cancer (NSCLC) xenografted subcutaneously onto nude mice can be effectively treated by the electrochemotherapy protocol using bleomycin or NCS and needle array electrodes. Other similar chemotherapeutic agents can also be effective using the method of the invention.

The results show a complete regression of Panc-3 tumors was achieved in 60% of the treated group with no palpable tumor seen even 77 days after the single treatment. Partial regression (80% reduction in tumor volume) was observed in 30% of cases, while only 10% did not respond (Table 2).

Histological studies clearly showed severe necrosis of the tumor region for the group subjected to ECT whereas no necrosis was apparent in the control group. Intratumoral drug injection with larger volume of bleomycin, combined with fanning to maximize uniform drug distribution throughout the tumor volume, was found to be very effective as compared to the conventional mode of injecting the drug prior to pulsing.

TABLE 2
Electrochemotherapy of Panc-3 with Bleomycin

Days after treatment	28	35	57	77
CR (100%)	6	6	6	6
PR (80%)	3			
NR (%)	1	1	1	1
Death				2*
Tumor regrowth		2		
Retreatment			2	
Histology		1		

Number of mice treated: 10

CR: Complete Regression

PR: Partial Regression

NR: No Response

*1 mice died after retreatment

1 mice died after 64 days survival

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

CLAIMS

1. An electrode apparatus for the application of electric fields to a selected portion of a living body, comprising:

support means;

5 an array of electrodes mounted on said support means in spaced relation to one another, at least one of said electrodes having a needle configuration for penetrating tissue for *in vivo* electroporation of cells of the tissue; and

an electric pulse generator for applying pulses of high amplitude electric signals to the electrodes proportionate to the distance between said electrodes for electroporation of cells between said electrodes.

10 2. An apparatus according to Claim 1 wherein said needle electrode having a cannula for the introduction of molecules into said tissue.

3. An apparatus according to Claim 2 wherein said array of electrodes comprises a central electrode of a first polarity and a plurality of electrodes of a second polarity encircling said central electrode.

15 4. An apparatus according to Claim 3 wherein said support means comprises a central tubular shaft, and said central electrode is adjustably mounted in said shaft and adjustably extendable from said shaft selectable lengths for selectable depths of penetration into tissue.

20 5. An apparatus according to Claim 4 wherein said support means comprises a collar mounted on said shaft and said electrodes are needles in a circular array supported on said collar.

6. An apparatus according to Claim 5 wherein said apparatus includes a rotary switch selectively positionable for connecting alternate opposite pairs of electrodes to said pulse generator.

7. An apparatus according to Claim 1 wherein said support means comprises a collar mounted on said shaft and said electrodes are a circular array of needles supported on said collar.

8. An apparatus according to Claim 1 wherein said array of electrodes
5 comprises a circular array of needle electrodes, and a switch assembly for selectively changing the polarity of opposing ones of said electrodes.

9. An apparatus according to Claim 7 wherein at least one of said needle electrodes has a cannula for injecting molecules into said tissue

10. An apparatus according to Claim 1 wherein said array of electrodes
10 comprises a linear array of needle electrodes, and said electrodes are adjustable relative to said support means in order to adjust a depth of penetration.

11. An apparatus according to Claim 1 wherein said array of electrodes
comprises a pair of spaced linear arrays of needle electrodes, and said arrays are
adjustable relative to one another on said support member, and includes means for
15 sensing a distance between said arrays.

12. An apparatus according to Claim 1 wherein said support means comprises a pair of tubular needles for inserting into selected tissue, and said electrodes are conductors insertable through said needles into said tissue.

13. An apparatus according to Claim 12 wherein said needles are removable
20 over said electrodes.

14. An apparatus according to Claim 7 wherein the field generator generates an electric field having a strength of between approximately 0.2 kV/cm and 20 kV/cm and between approximately one pulse and one hundred pulses for application to a tissue.

15. Use of electroporation for introducing molecules into cells, comprising:
providing an array of electrodes, at least one of said electrodes having a needle
configuration for penetrating tissue;

5 inserting said needle electrode into selected tissue for introducing molecules into
the tissue;

positioning a second electrode of said array of electrodes in conductive relation
to said selected tissue so that said tissue is between said first and second electrodes;

providing an electric pulse generator;

connecting said electric pulse generator to said electrodes; and

10 operating said electric pulse generator for applying pulses of high amplitude
electric signals to the electrodes proportionate to the distance between said electrodes for
electroporation of cells of the tissue.

16. The use of Claim 15 wherein said step of providing said array of
electrodes comprises providing first and second needle electrodes comprising a pair of
15 tubular needles for inserting into selected tissue, and said electrodes are separable
conductors insertable through said needles into said tissue.

17. The use of Claim 16 wherein said needles are removable over said
electrodes.

18. The use of Claim 15 wherein said step of providing said array of
20 electrodes comprises providing a central electrode of a first polarity and a plurality of
electrodes of a second polarity encircling said central electrode.

19. The use of Claim 18 comprising the step of providing a switch assembly
including a rotary switch selectively positionable for connecting alternate opposite pairs
of electrodes to said pulse generator.

25 20. The use of Claim 15 wherein the molecule is selected from the group
consisting of a chemotherapeutic agent, a polynucleotide and a polypeptide.

21. The use of Claim 20 wherein the chemotherapeutic agent is bleomycin.

22. The use of Claim 15 wherein the tissue is selected from the group consisting of pancreas, lung, heart, kidney, muscle, breast, colon, prostate, thymus, testis, and ovary.

5 23. The use of Claim 15 wherein said step of providing said array of electrodes comprises providing an array of multiple needle electrodes, comprising a plurality of opposed pairs of needle electrodes for inserting into selected tissue, and said step of applying pulses to said electrodes including selectively applying pulses to opposing pairs of electrodes.

10 24. The use of Claim 23 wherein said step of applying pulses to said array of electrodes comprises applying said pulses sequently to pairs of electrodes in said array.

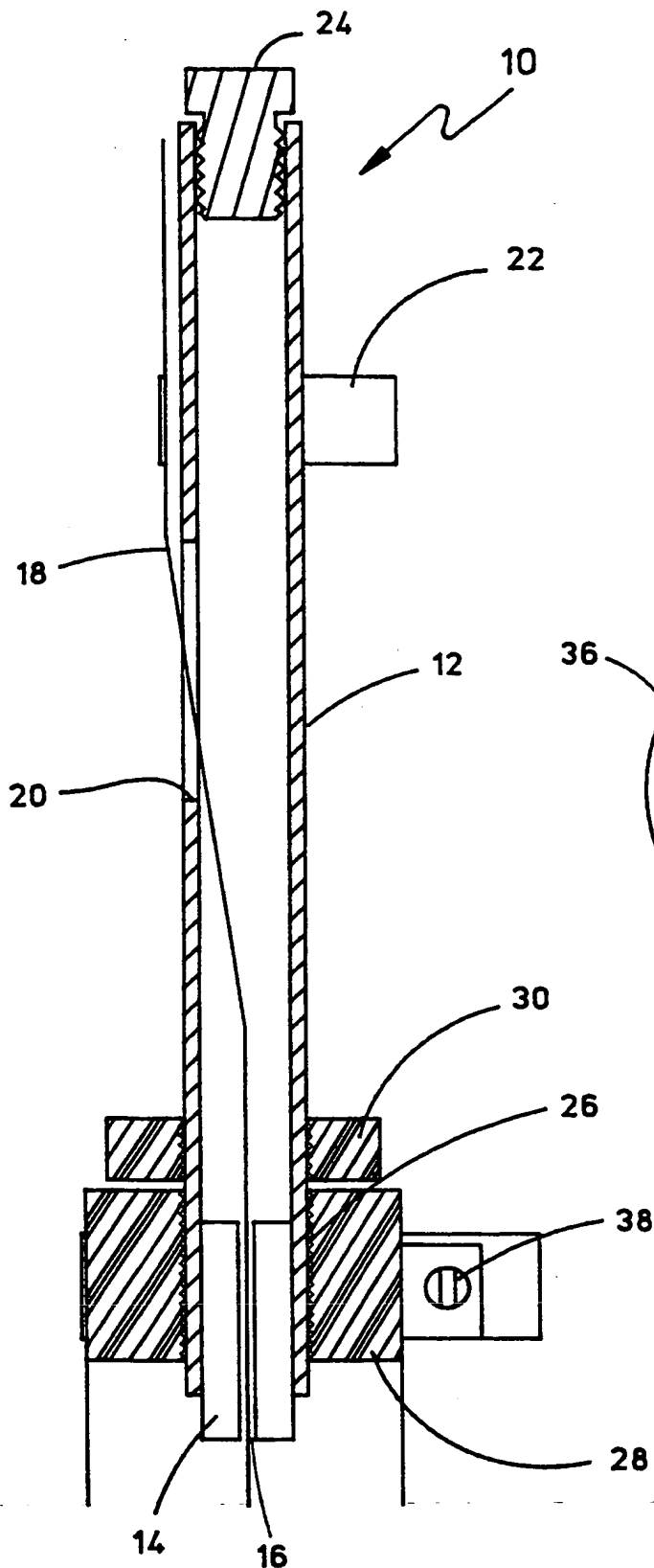


FIG. 1

SUBSTITUTE SHEET (RULE 26)

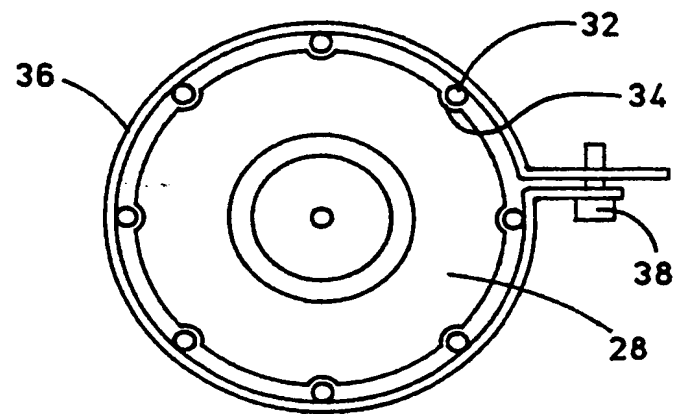


FIG. 2

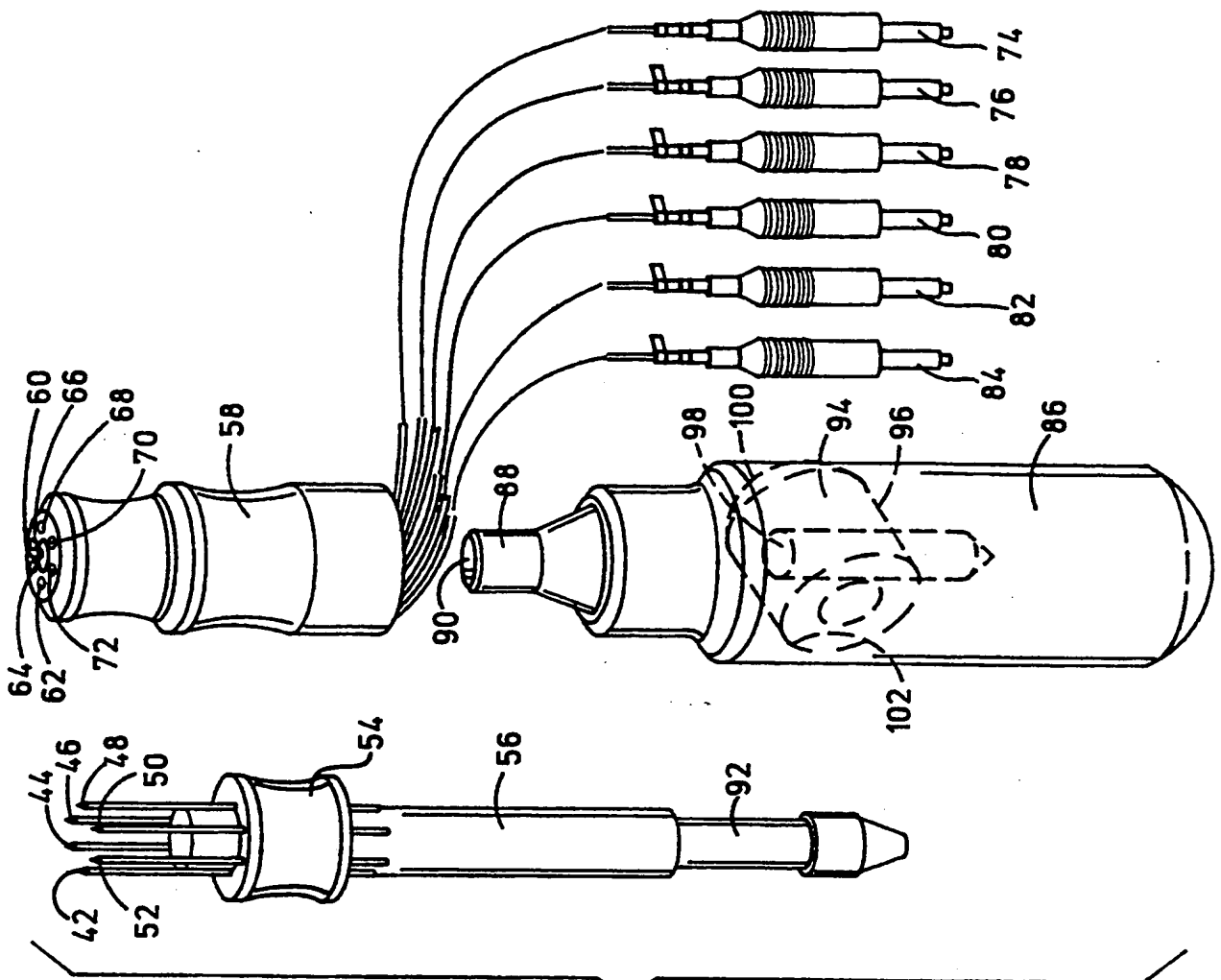


FIG. 3

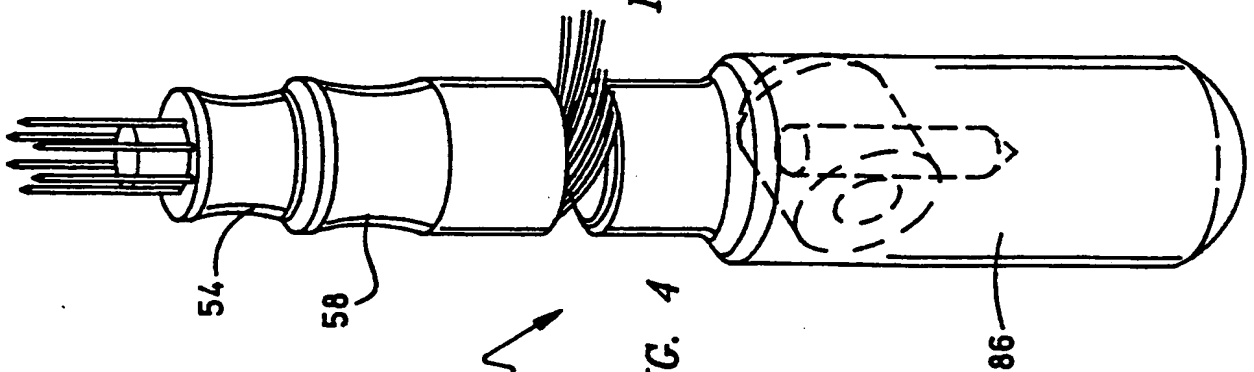
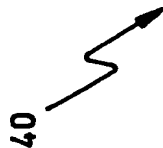


FIG. 4



SUBSTITUTE SHEET (RULE 26)

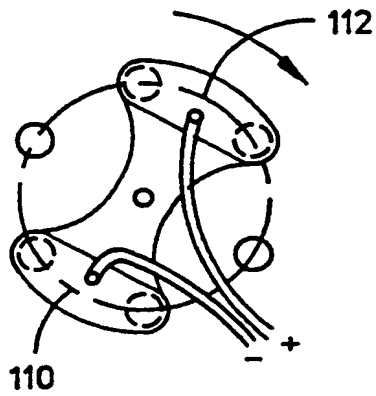


FIG. 6a

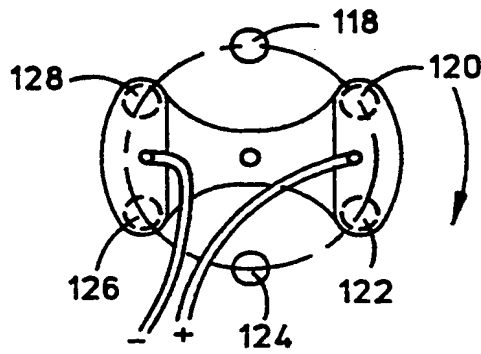


FIG. 6b

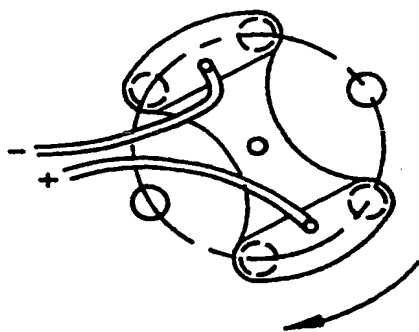


FIG. 6c

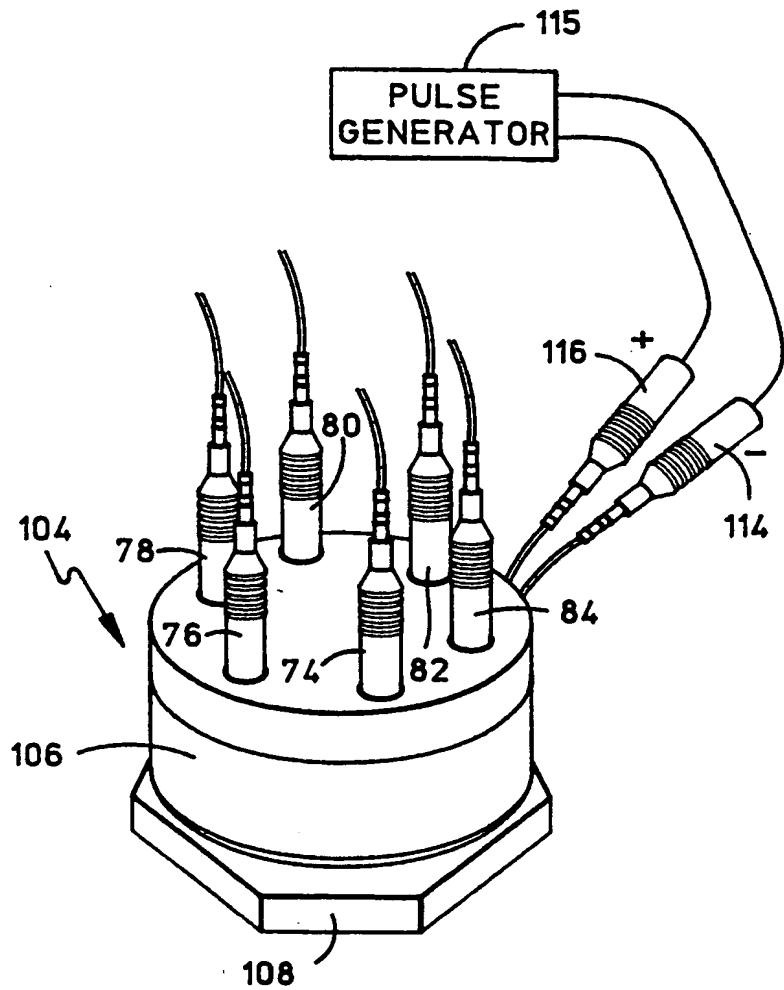


FIG. 5

4/16

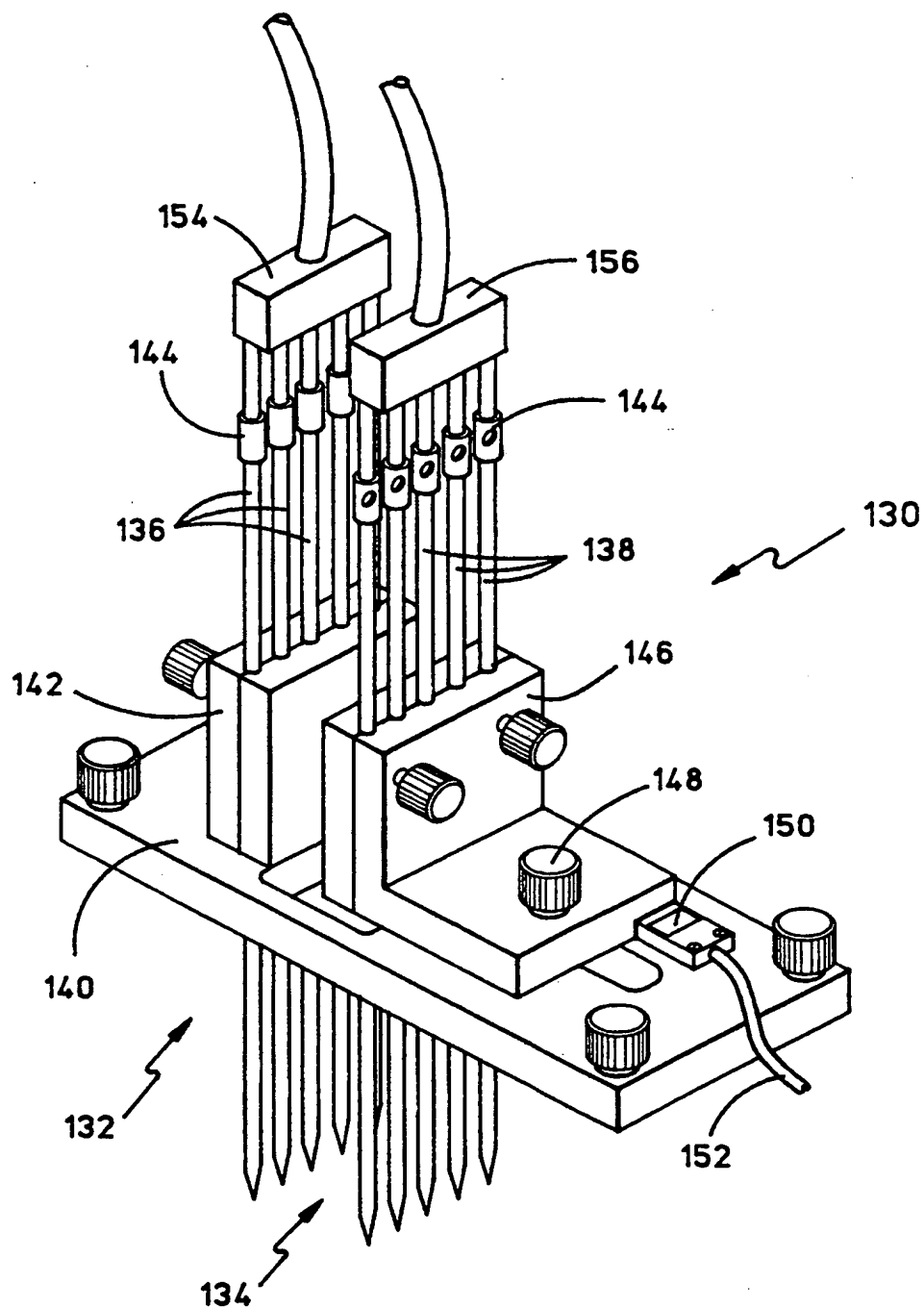
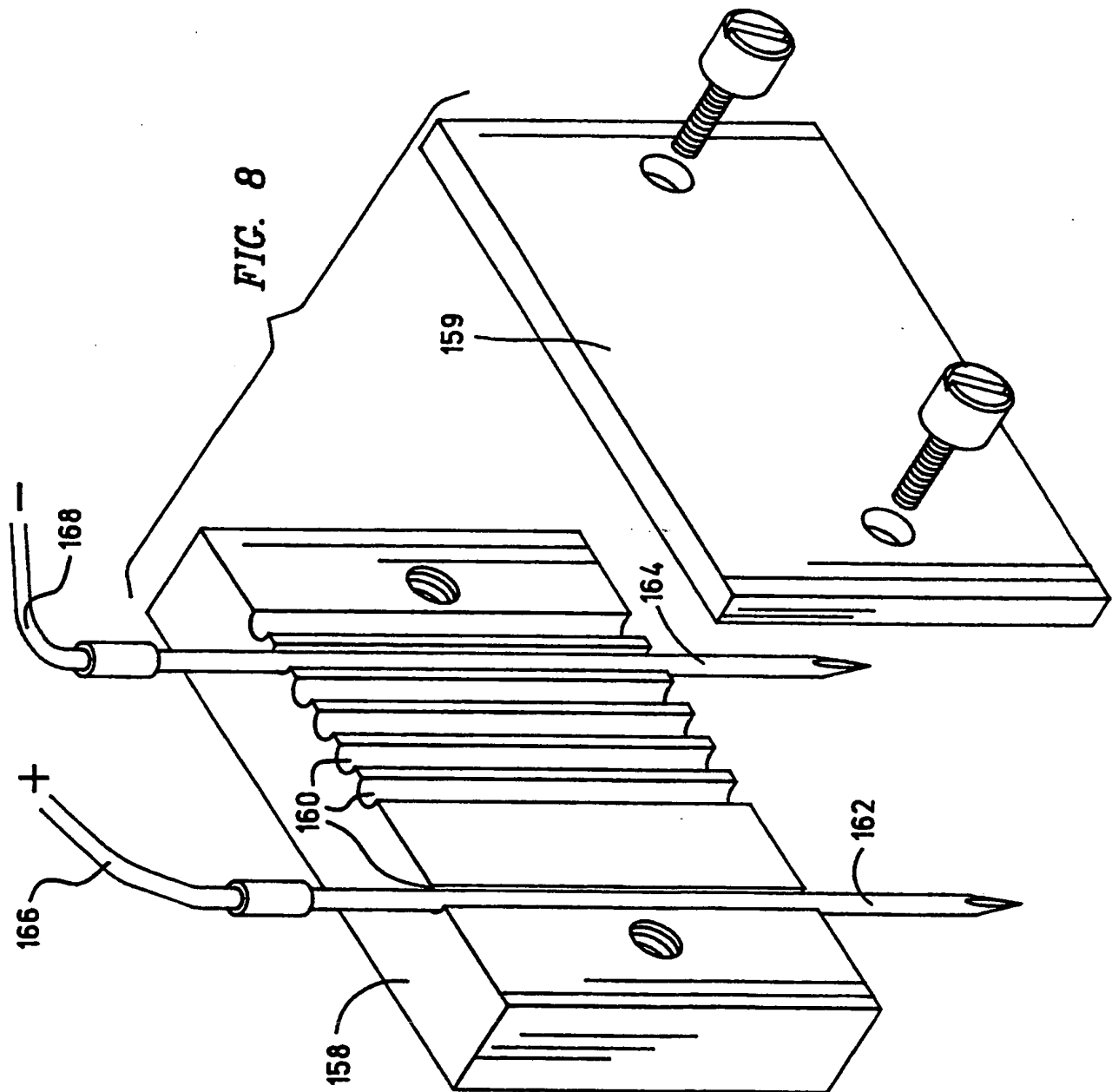


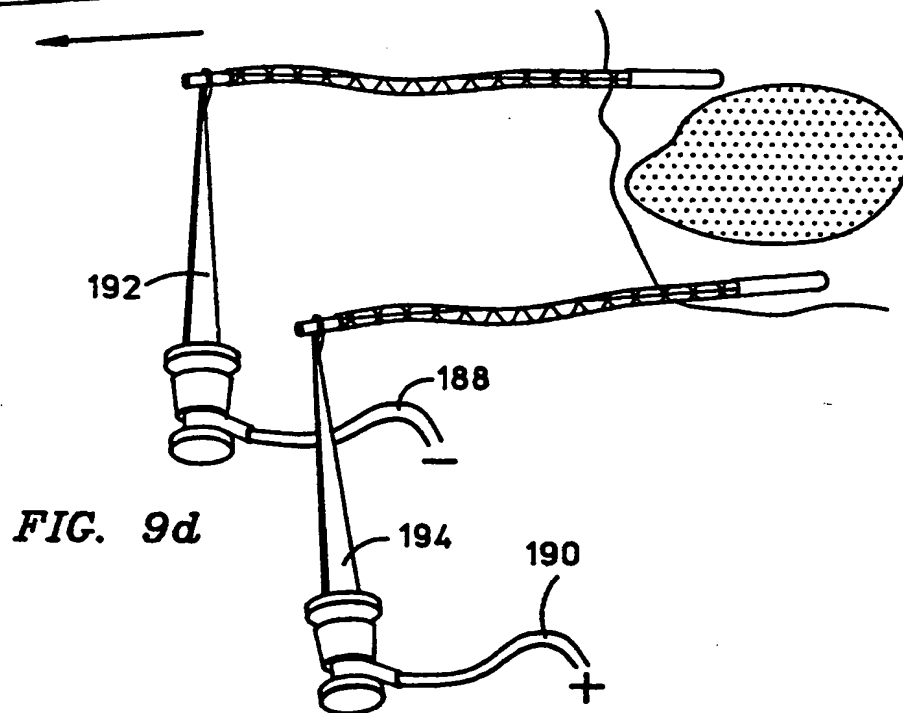
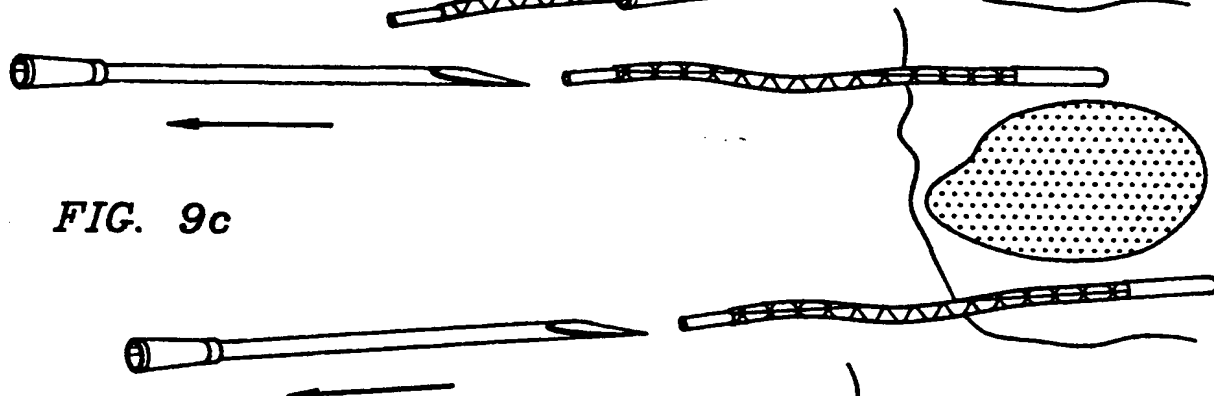
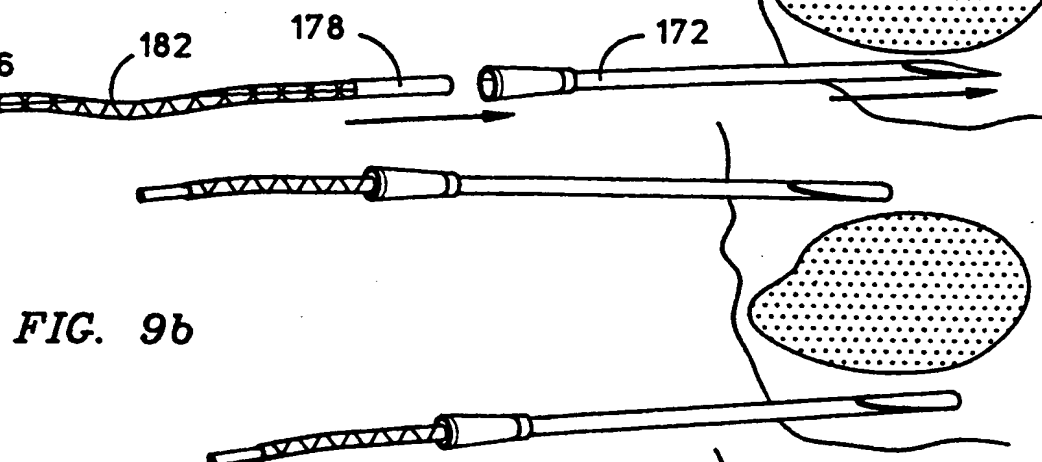
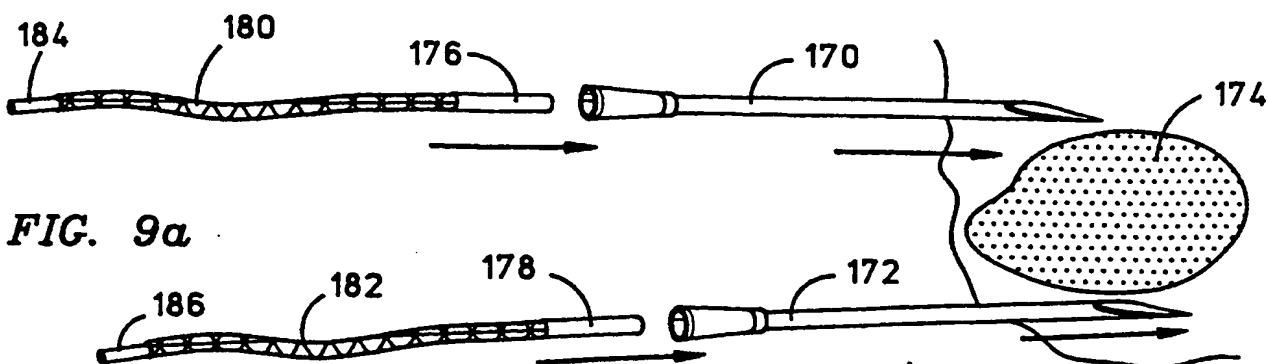
FIG. 7

SUBSTITUTE SHEET (RULE 26)



SUBSTITUTE SHEET (RULE 26)

6/16



SUBSTITUTE SHEET (RULE 26)

7/16

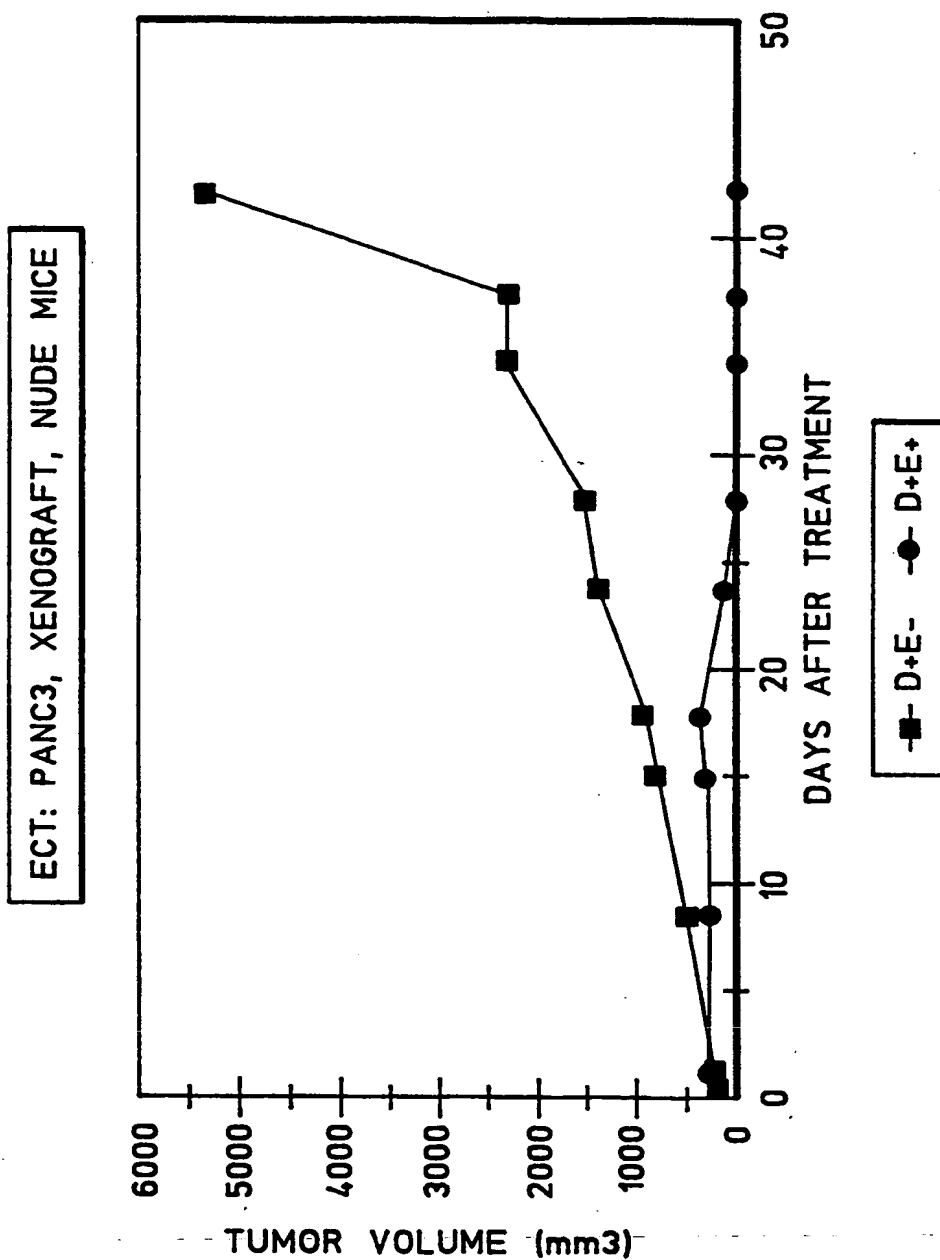


FIG. 10a

SUBSTITUTE SHEET (RULE 26)

8/16

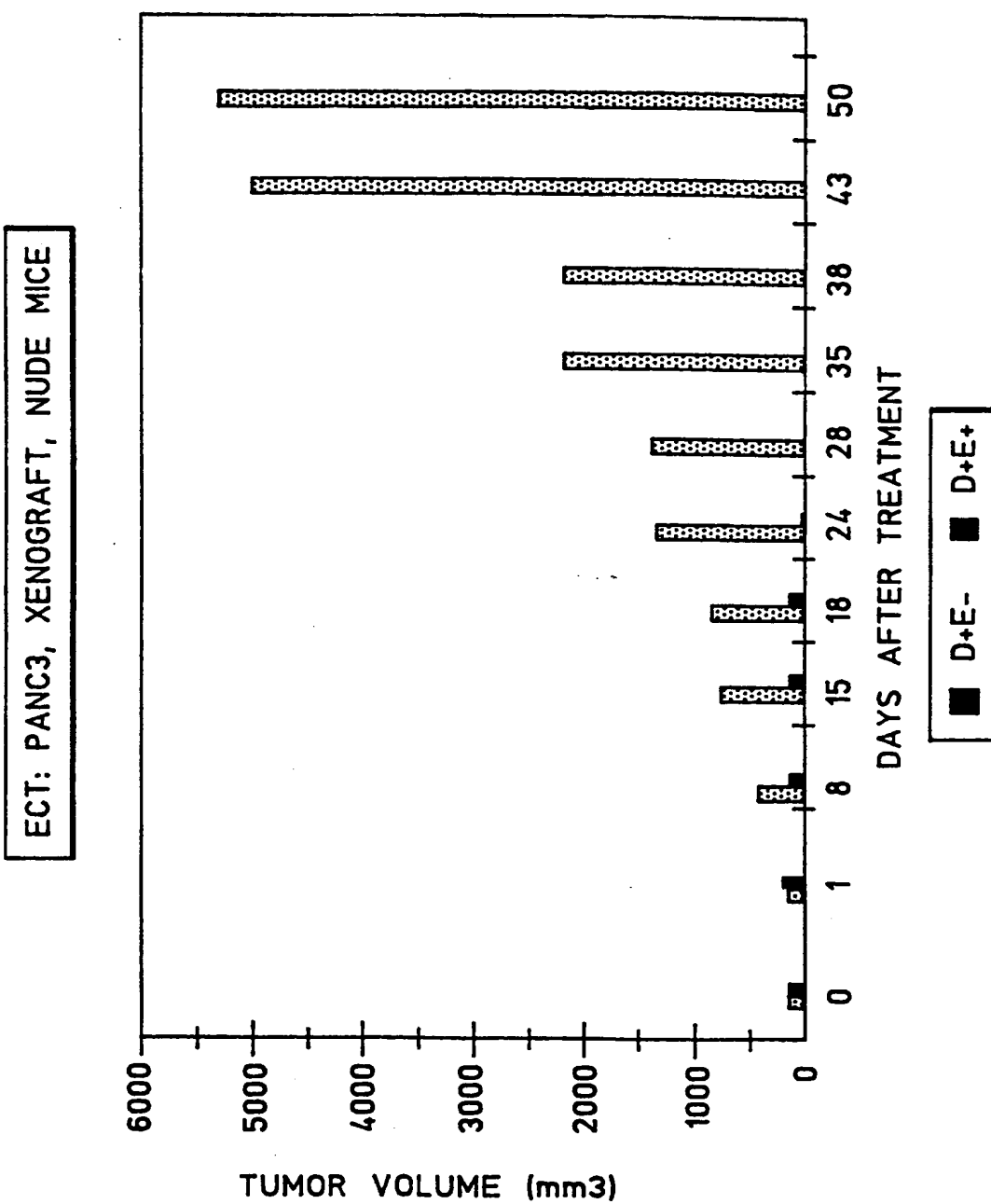


FIG. 10b

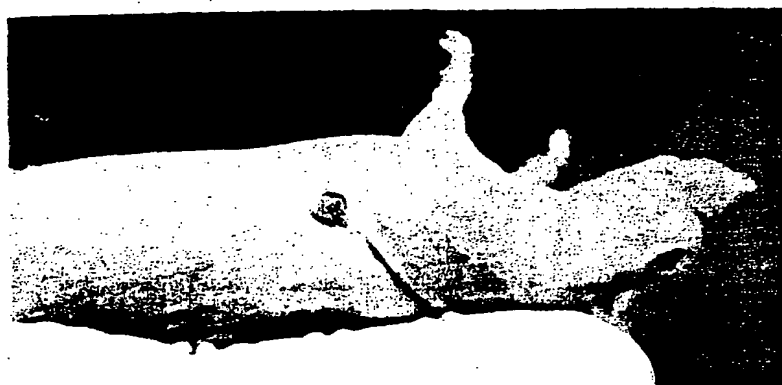
**TUMOR GROWTH OF PANC3 AFTER
ELECTROCHEMOTHERAPY WITH BLEOMYCIN**



**CONTROL (D+E-)
AFTER 29 DAYS**



**TREATED (D+E+)
AFTER 9 DAYS**



**TREATED (D+E)
AFTER 29 DAYS**



**TREATED (D+E+)
AFTER 46 DAYS**

FIG. 11

SUBSTITUTE SHEET (RULE 26)

10/16

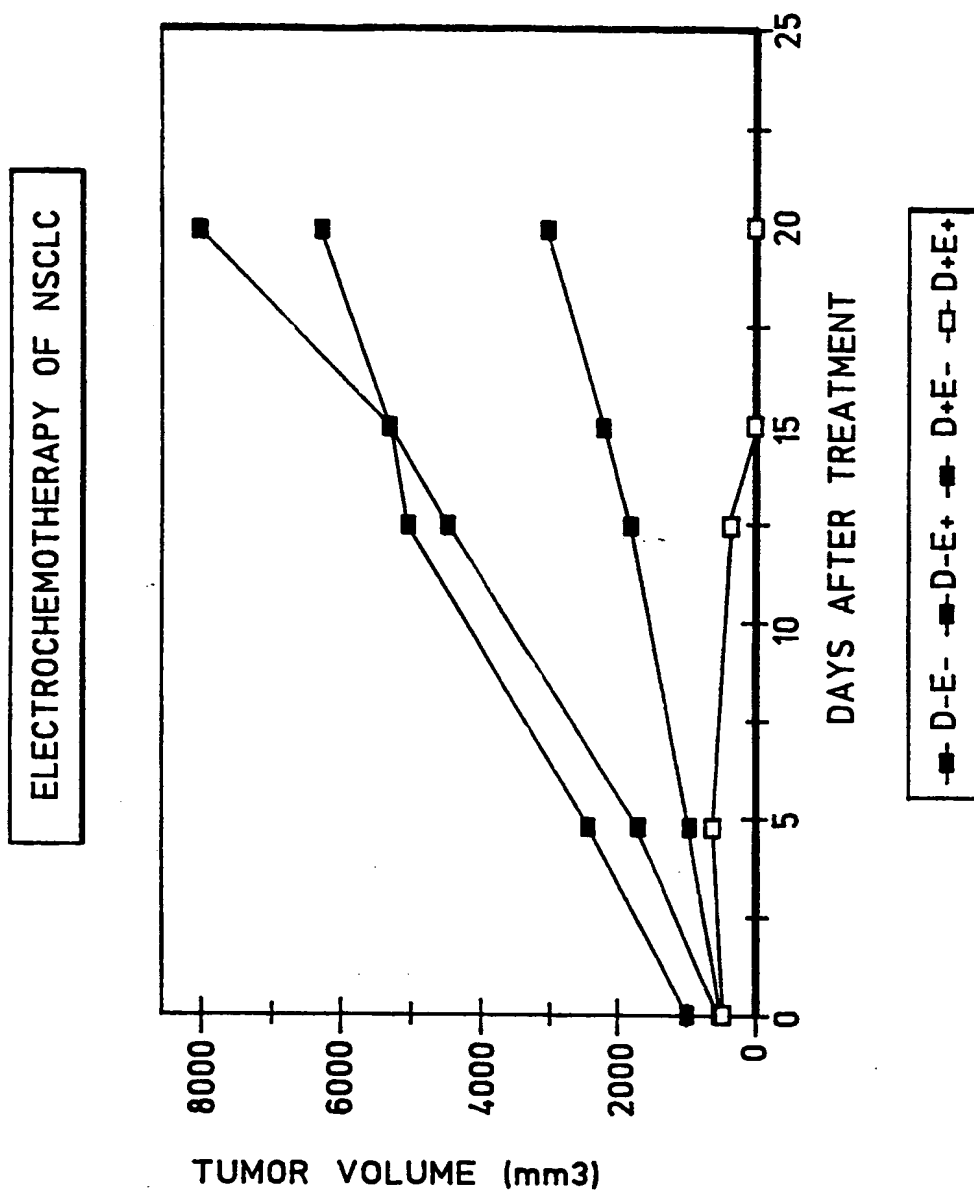


FIG. 12a

SUBSTITUTE SHEET (RULE 26)

11/16

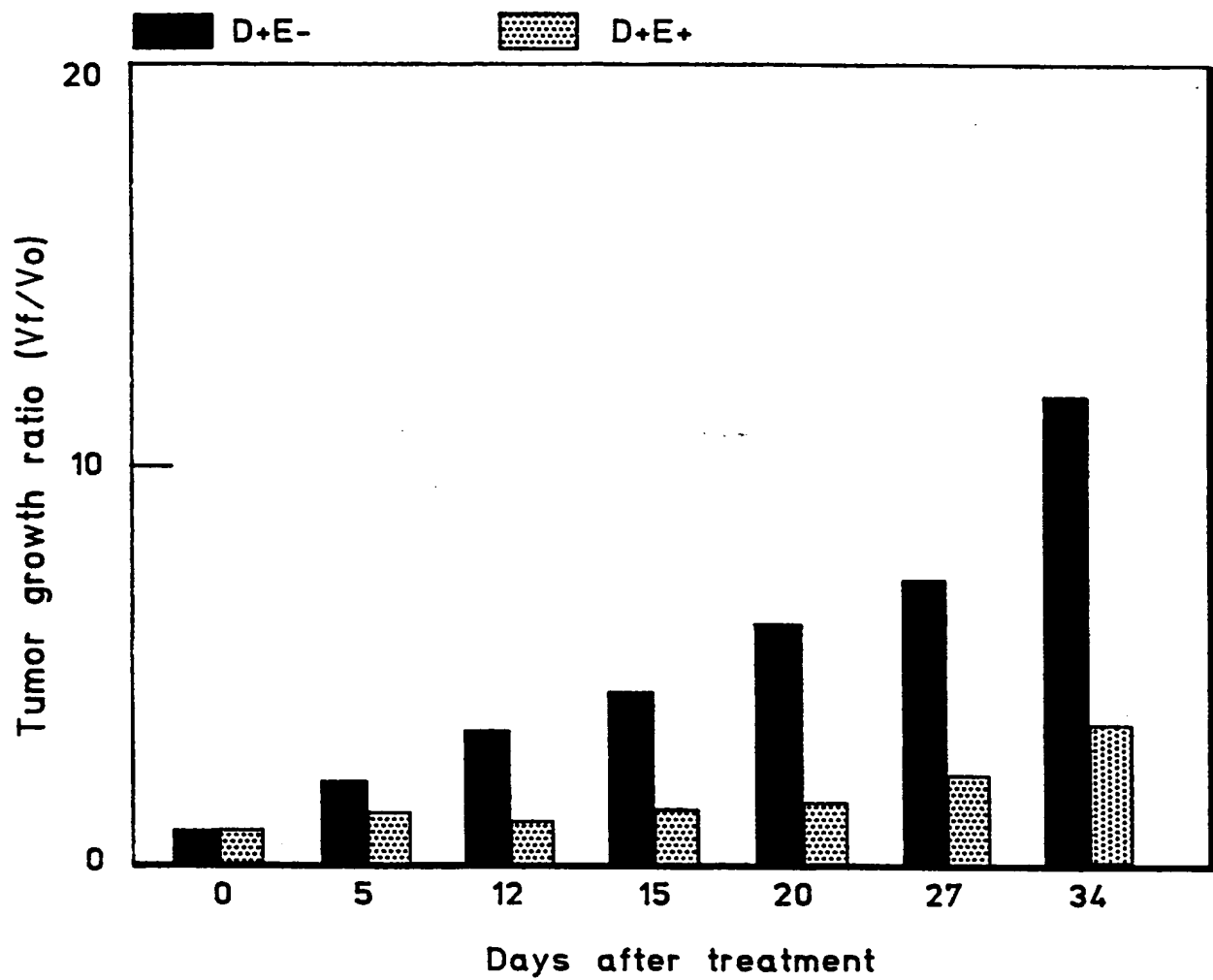
ECT of NSCLC with BLM
1000 V/cm

FIG. 12b

SUBSTITUTE SHEET (RULE 26)

12/16

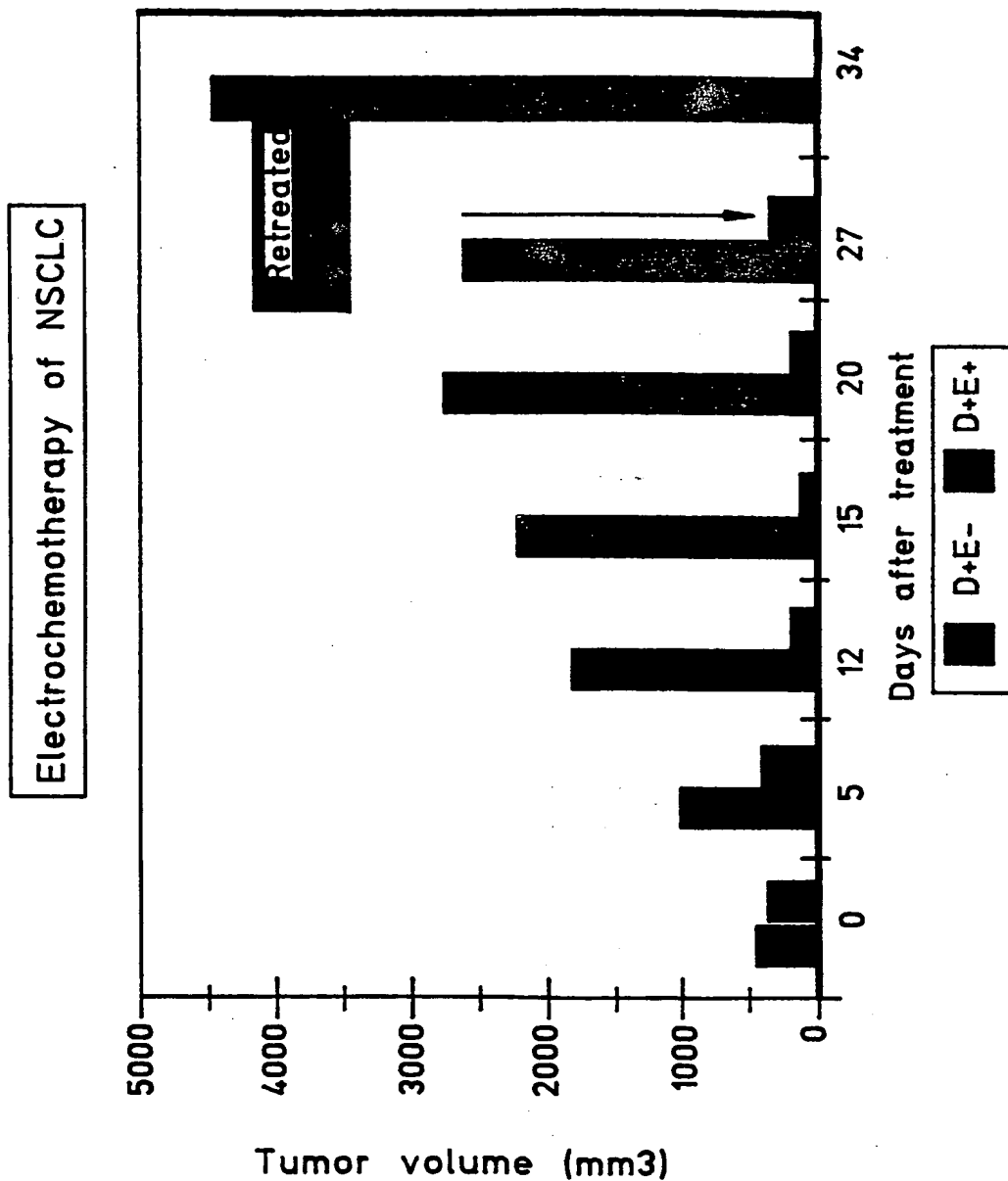
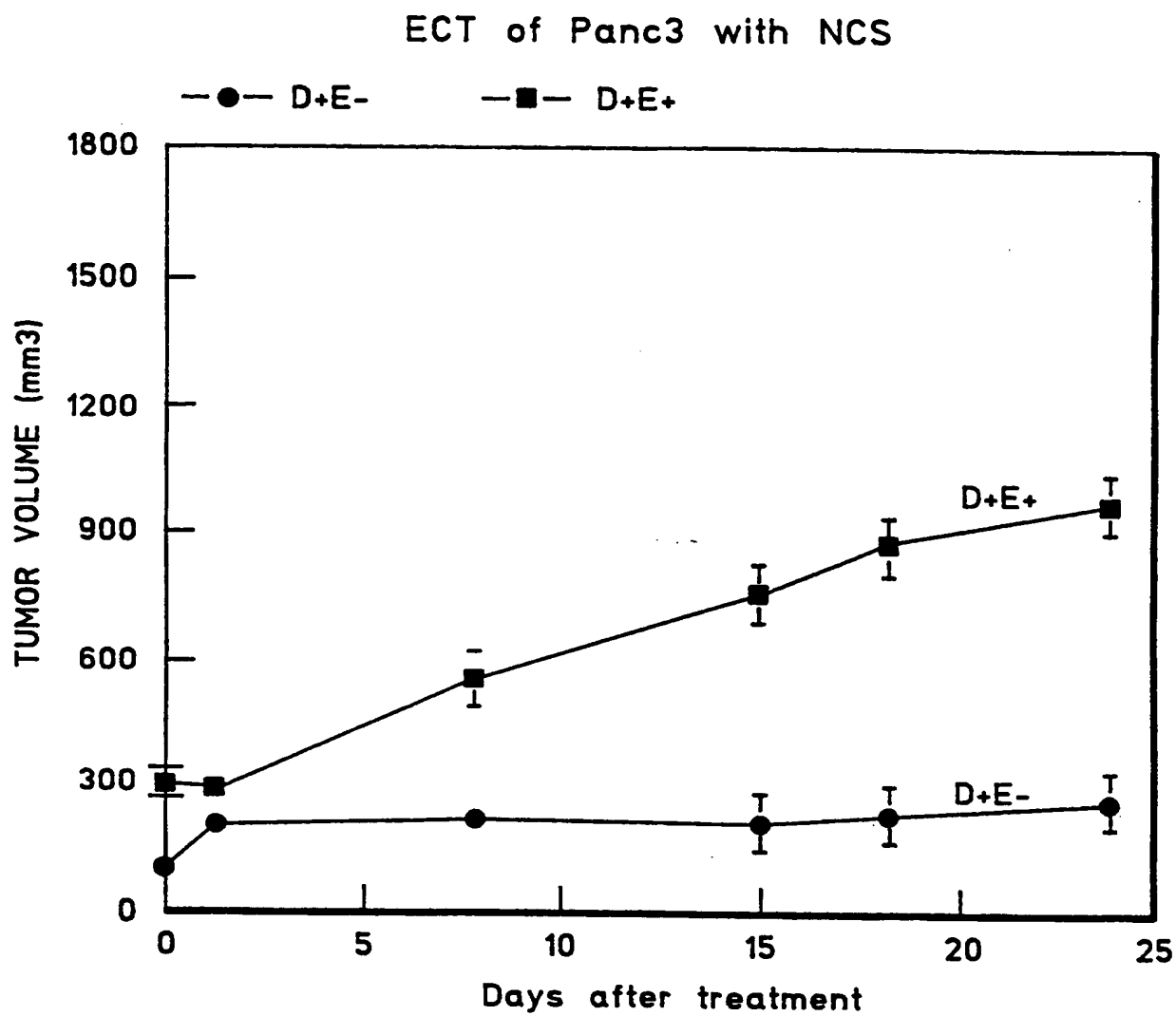


FIG. 13

SUBSTITUTE SHEET (RULE 26)

13/16

*FIG. 14a*

SUBSTITUTE SHEET (RULE 26)

14/16

ECT of NSCLC with NCS

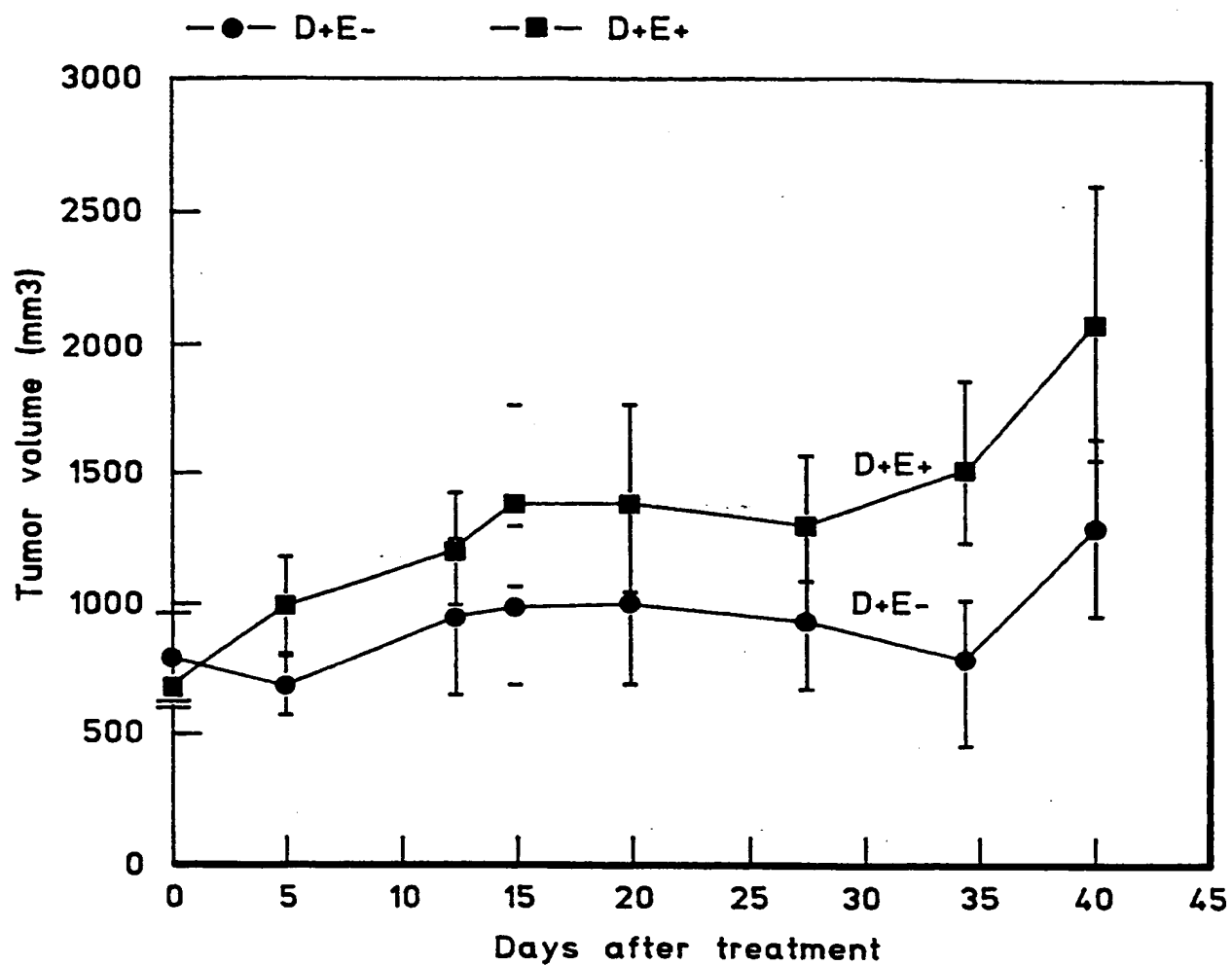


FIG. 14b

SUBSTITUTE SHEET (RULE 26)

ECT of Panc3 with NCS (PP)

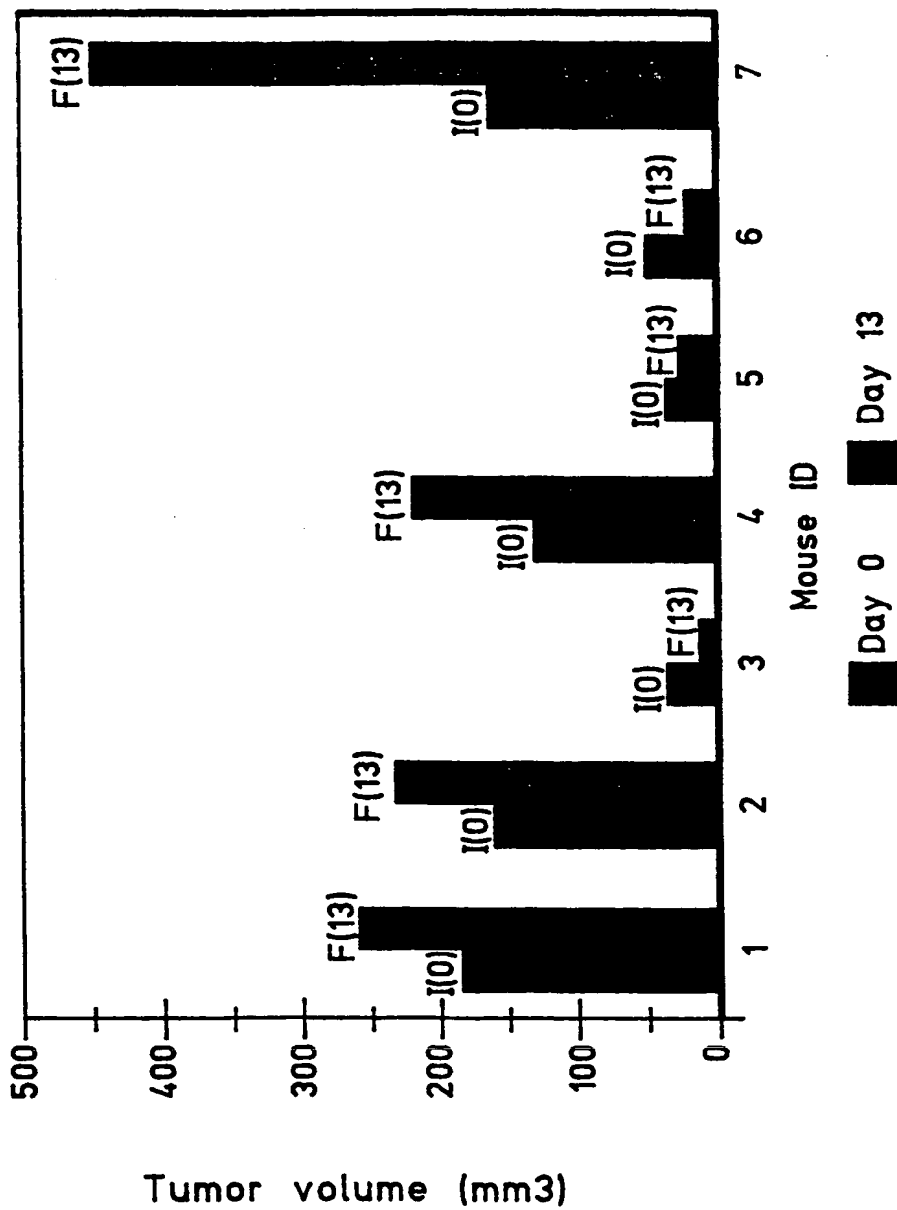


FIG. 14c

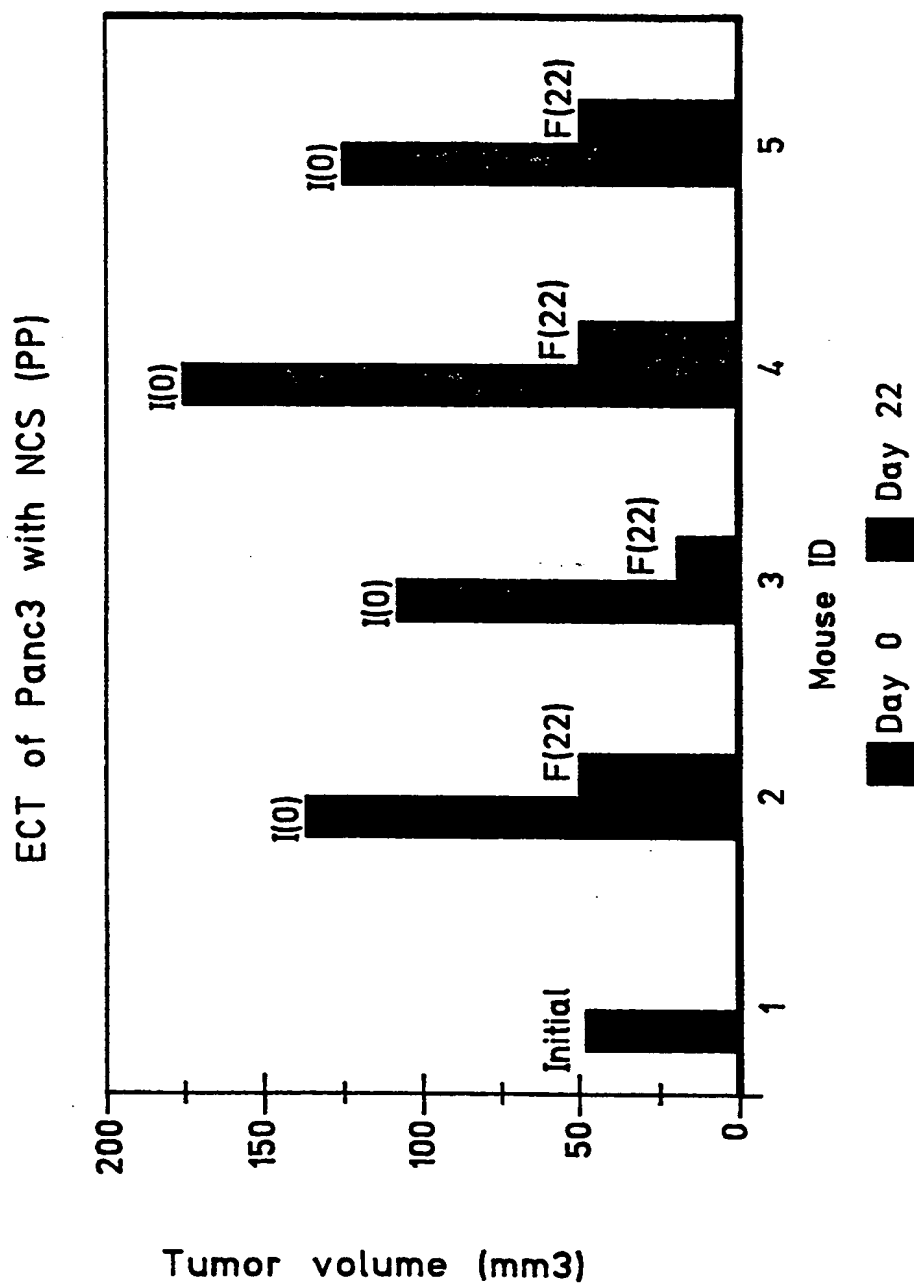


FIG. 14d

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/07470

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61N1/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 22526 (CENTRE NAT RECH SCIENT ;MIR LLUIS M (FR); ORLOWSKI STEPHANE (FR);) 13 October 1994	1-3,7-9, 12-18,20
A	see page 5, line 14 - page 11, line 26; figures	4,5,10, 21-24
A	DE,A,863 111 (WALTER HALLEGGER) 15 January 1953 see page 1, line 32 - page 3, line 41; figures	1-4,9, 12-17
A	EP,A,0 378 132 (TOMAS JUSTRIBO JOSE RAMON) 18 July 1990 see the whole document	1,2,9, 10,12-17
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

- * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * "&" document member of the same patent family

Date of the actual completion of the international search

10 September 1996

Date of mailing of the international search report

27.09.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Rakotondrajaona, C

INTERNATIONAL SEARCH REPORT

Inter: al Application No
PCT/US 96/07470

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 116 238 (PETTIJOHN DAVID) 26 September 1978 see column 2, line 34 - column 3, line 58; figures ---	1,2,9,15
A	WO,A,93 17754 (ELAN MEDICAL TECHNOLOGIES) 16 September 1993 see page 8, line 35 - page 10, line 15; figures -----	1,15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/07470

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9422526	13-10-94	FR-A- 2703253 AU-A- 6379494 EP-A- 0693951	07-10-94 24-10-94 31-01-96
DE-A-863111		NONE	
EP-A-0378132	18-07-90	NONE	
US-A-4116238	26-09-78	JP-A- 53024550	07-03-78
WO-A-9317754	16-09-93	US-A- 5279544 EP-A- 0630276 JP-T- 7508427 US-A- 5527288 ZA-A- 9301775	18-01-94 28-12-94 21-09-95 18-06-96 30-09-93